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Use of Starling Nest Boxes for Field Reproductive Studies Provisional Guidance Document and Technical Support Document

Huxley Coll. of Environmental Studies, Bellingham, WA

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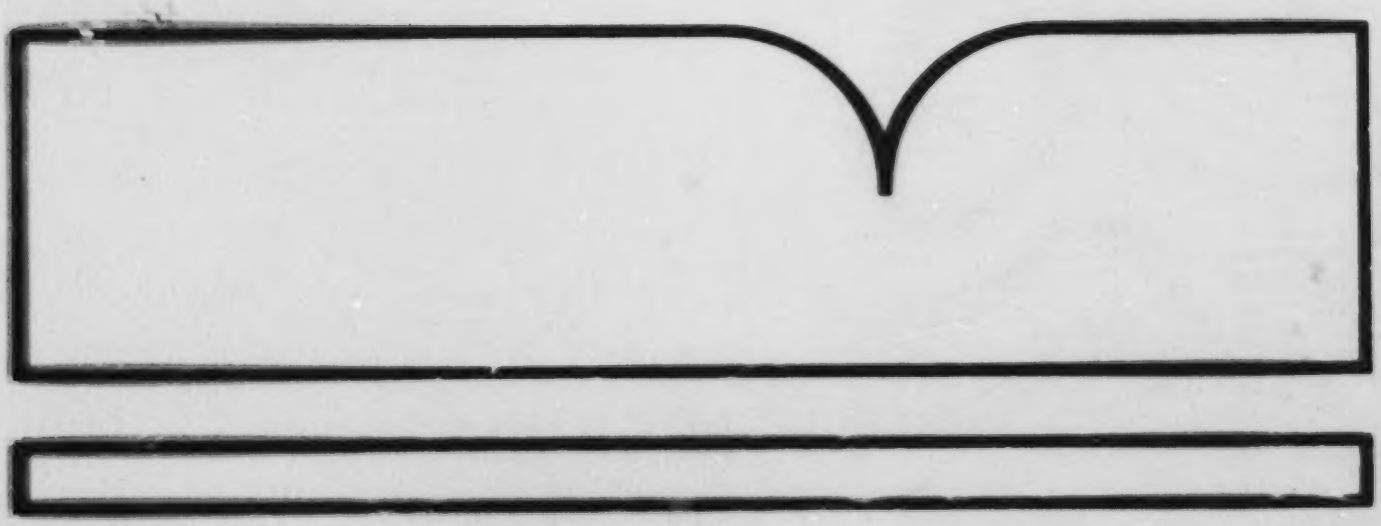

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THE USE OF STARLING NEST BOXES FOR FIELD REPRODUCTIVE STUDIES:

PROVISIONAL GUIDANCE DOCUMENT AND TECHNICAL SUPPORT DOCUMENT

Submitted By:

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16. ABSTRACT

The purpose of this report is to propose nest box field testing guidelines for determining reproductive effects of agricultural pesticides on European starlings (Sturnus vulgaris), and provide backgroud information and justification for the procedures and methodology described. Investigation of the effects of pesticides on birds has generally been limited to either laboratory tests of pen-reared species or to small-scale studies of captive birds. Although much useful information has been derived from these studies there exists a relative paucity of literature regarding the effects of pesticides on free-ranging birds under typical pesticide use conditions. Previous studies have focused mainly on gallinaceous and/or waterfowl species. The information on the toxicity of various compounds to these species has been extrapolated to other bird species, inherently introducing substancial amount of potential error. In order to reduce this error, it is necessary to expand the number of species tested and vary the conditions under which the testing occurs.

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PROVISIONAL GUIDANCE DOCUMENT FOR CONDUCTING A STARLING NEST BOX STUDY

1.0 PURPOSE

The purpose of this report is to propose nest box field testing guidelines for determining reproductive effects of agricultural pesticides on European starlings (Sturnus vulgaris). An explanation of specific recommendations in this report can be found in the accompanying report entitled: Technical Support Document for Starling Nest Box Study.

2.0 JUSTIFICATION

The ability to assess potential impacts of pesticides on avian species is of considerable concern and controversy. The testing of pesticides has become markedly more rigorous during the last two decades, yet there still exists a substantial need to improve the efficacy, applicability, and breadth of toxicity testing. Pesticide tests have been primarily conducted in the laboratory or in controlled environmental chambers. Testing guidelines have been established for most laboratory studies of pen-reared species, but in the past there have not been any defined guidelines for conducting large-scale field studies. The United States Environmental Protection Agency has recently published a Guidance Document for Terrestrial Field Studies (Fite et al., 1988). This document describes the general means through which a pesticide test can be designed and conducted. Although this guidance document provides a useful framework for the design of field studies, there is a paucity of specific or standardized testing procedures. There is a need to define and incorporate standardized methods into a large-scale field test.

The interpretation of data acquired from field studies may be characterized by a high degree of uncertainty. Variability between sample units are generally high and the ability to extract meaningful conclusions from such studies is often compromised. Well designed field studies that have standardized procedures can reduce some of the variability typically associated with field study data sets. Starling nest boxes, as one example, can be used to reduce uncertainty by providing a large number of subsamples for each sample unit. By increasing the number of values per sample unit (study site), and by the use of an appropriate number of replicate study sites, the inherent high degree of variability in field studies can be reduced. Reducing variability in field experiments is a necessary first step in improving the reliability and verifiability of pesticide field studies.

Nest boxes for starlings represent one means of introducing standardized testing procedures into a pesticide field study. Nest boxes can be used to encourage starlings to nest within a study site. The establishment of a local population of starlings provides researchers with a large number of accessible nests from which pesticide-induced perturbations can be documented. Starlings occupying nest boxes can be used to collect information on reproductive, behavioral, and biochemical parameters. Starling nest box studies are particularly useful because of the ease and consistency with which data can be acquired. Frequent observations of nest box contents allows investigators to collect detailed information on all aspects of starling reproduction.

The original purpose for the development of a starling nest box field test arose from a request by the USEPA to establish a field testing procedure to assess the effects of pesticides on songbirds. The starling has many attributes that allow for data collection that can contribute information for evaluation of the effects of pesticides on songbirds. In this provisional guidance document and the technical support document we describe procedures

necessary for a starling nest box field study. We also discuss the positive and negative aspects of using starling nest boxes as a standardized field testing technique.

3.0 DISCLAIMER

It is not the intent of this document to suggest or imply that starlings are an indicator species or that starling nest box studies are independently the optimal means of determining pesticide effects on songbirds. Such an implication regarding the use of any single species as an 'indicator species' which can be used as a means of extrapolating biological effects to other species is premature given our current understanding of wildlife ecotoxicology. Valid field testing procedures for determining population level impacts of pesticides must include a panel of different species representing a variety of avian morphological, behavioral, physiological, and biochemical characteristics as well as diverse biogeographical regions. This document describes the procedures for a method that can be standardized and incorporated into full-scale avian field tests.

The decision to use starlings as a test species is based upon criteria set forth in Appendix 1 "Selection of the Starling as a Test Species". The provisional guidance document is based upon the aforementioned criteria and is a detailed description of procedures which can be used to effectively conduct a starling nest box study. Furthermore, this document describes the means through which effect(s) on starling reproductive success resulting from an application of an agricultural pesticide can be recorded and analyzed. It is not the intent of this provisional guidance document to suggest that the methods described herein are the only way in which to conduct a starling nest box study. We have presented a very specific set of guidelines in which each defined procedure is based upon information from 1) an extensive survey of available literature pertaining to starlings, nest box studies,

and wildlife toxicology; 2) a two-year starling nest box study; and 3) our best scientific judgement. It is the responsibility of investigators to either follow the guidelines described in this document or to modify the procedures in such a way as to derive a superior experimental design. The final experimental design should effectively address a series of well defined questions regarding the reproductive effects upon starlings resulting from exposure to a specific pesticide formulation and use pattern. It is our hope that the procedures defined in this document will assist future investigators in achieving this goal.

5.0 TEST PROCEDURE

5.1 General Overview

This document describes a three-year full scale field testing procedure for the determination of reproductive effects of agricultural pesticides on European starlings (hereafter referred to as starlings). The first field season will serve as a pilot study in which occupancy of boxes and reproductive parameters will be documented. Information from the pilot study will be used to improve the experimental design for years two and three. During the pilot study no pesticide will be applied to the test sites. The pesticide will only be applied during the year-two and year-three studies.

The testing procedure involves 1) establishing local starling populations in agricultural fields by erecting 30 starling nest boxes per field (prior to the breeding season) and allowing starlings the opportunity to occupy the nest boxes; 2) monitoring the nest boxes throughout the breeding season; 3) applying an agricultural pesticide, blank granules, or a carrier solvent, to the study sites (during years two and three); 4) collecting reproductive data on the starlings; and 5) comparing the data between and within each treatment group.

5.2 Goal of Study

The United States Environmental Protection Agency (USEPA) is directed by the amended Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, P.L. 92-516) to ensure that pesticide usage will not cause unreasonable adverse effects on the environment (including nontarget wildlife species). According to guidelines stated in Subdivision E of the Pesticide Assessment Guidelines, the USEPA may require pesticide registrants to design and implement field tests which can detect a 20% difference in a

given avian reproductive parameter between treatment and control fields. The USEPA has set acceptable error limits for both a Type I error (alpha error: rejecting a true null hypothesis) and a Type II error (beta error: accepting a false null hypothesis). The acceptable levels for each of these errors are: Type I alpha = 0.05 and for Type II beta = 0.2.

The primary goal of this provisional starling nest box guidance document is to provide a detailed description of the procedures necessary to conduct a starling nest box study. Although there are numerous endpoints that should be investigated in pesticide field studies, the number of fledgings per field is the primary reproductive parameter to document. The number of fledglings per field will be compared between treatment and control fields. A significant difference between treatment groups in this parameter indicates the need to compare other reproductive parameters between treatment groups before pesticide-induced effects can be established. A verifiable decrease in reproductive parameters of greater than, or equal to, 20% among the treatment fields as compared to control field values suggests that the pesticide of concern had an adverse effect on starling reproduction and that it may cause reproductive perturbations in other birds.

In addition to examining the number of fledglings per field, the starling nest box study will provide sufficient information so that the aspects of starling reproduction which are affected by the chemical can be identified. Other starling reproductive parameters (e.g., the number of nestlings per field, nestlings per nest per field, nestling mortalities per field, fledglings per nest per field, eggs per nest per field, the percent fledgling success per field, or the mean weight of 16-day-old nestlings per nest per field) should be analyzed to identify those parameters which were altered by the pesticide. If the number of fledglings per field is not significantly different between fields, then the

investigators should have sufficient information to verify that 1) the test maintained sufficient power; 2) the test was properly executed; 3) some reduction in reproductive output did or did not occur; 4) some mortality did or did not occur; and 5) there were not any identifiable differences in any of the parameters measured between fields. A thorough description of the reproductive parameters on all fields will be valuable whether or not a significant treatment effect is observed.

The information derived from starling nest box studies should be used in conjunction with other laboratory and field studies before a pesticide formulation and its proposed, or current, use pattern are determined not to have an unreasonable adverse effect on birds.

5.3 Number of Fields

5.3.1 Number of Fields for the Year-one Study

In an effort to decrease variability between paired fields (blocks), and to improve the design for study years two and three of the project, investigators should select eight pairs of fields for the year-one study. Following selection of the fields, 30 nest boxes should be erected in each of the fields. Starlings should then be allowed to occupy the nest boxes and data on reproduction should be collected according to the schedule described under Section 5.9 (Monitoring Schedule of Nest Boxes). During this first year of study no pesticide would be applied to the fields. The purpose of the first year of study is to 1) verify that starling reproductive parameters were similar in value and variance within blocks; 2) allow starlings one year to locate nest box sites so that occupancy rates should be higher during the

second year of the project; 3) select six pairs of fields (from the eight studied) to be used for the year-two and year-three studies; and 4) change the location of the study in the event that occupancy rates are too low in the selected field sites.

This first year of study is essential in order to implement an effective longer term study. When conducting any field study it is critical that the amount of inherent variability in the parameter being investigated be established. The number of replicates necessary to maintain the power of a test is dependent upon the expected variability. The information from year-one will provide investigators with a means of identifying the variability in starling reproductive parameters for the areas of study and will therefore allow them to more accurately assign paired fields.

5.3.2 Number of Fields for the Year-two and Year-three studies

Six pairs of agricultural fields will be necessary for the study (refer to support document for explanation and justification). A paired design is used to limit the total number of fields required for the study. The paired field design is in effect a randomized blocks design in which each block contains a treatment and a control field (Fite et al., 1988). The selection of fields to be paired together should be based upon known starling use of the field, surrounding habitat, and also upon maximizing the number of physical similarities between the fields. By maximizing the similarities between the fields the investigator reduces the variability between fields and increases the chance of identifying treatment effects.

5.4 Field Site Selection

5.4.1 Field Characteristics

The selection of field sites is dependent upon the pesticide to be tested and the type of crop(s) to which it will be applied. Because most pesticides will be applied to a variety of crops, the selection of field types to be used for this test should be those that are used most frequently by starlings. Starlings are typically grassland feeders and, if food availability remains adequate, they will forage almost entirely in a grassland during the breeding season. Starlings will, however, feed in other agricultural fields (e.g., corn and peas) if there is an accessible food supply. Therefore, if a pesticide is registered or is proposed for use on grasslands (e.g., hay fields) then grasslands should be selected in preference over other field types. If the pesticide will not be applied to grasslands, then fields which have the greatest likelihood of being used by starlings should be selected.

The type of fields lying adjacent to study plots is also a very important factor to consider when selecting fields for a pesticide study. The relative quality, availability, and type of food present on a study site and adjacent fields will influence the amount of time a starling forages in the study area. Study sites that have a variety, and abundance, of ground dwelling invertebrates will probably be extensively used for foraging by resident starlings. Study sites that have few varieties or sparcely distributed soil invertebrates, and are adjacent to fields with a larger and more diverse population of invertebrates, will be used less frequently by adult starlings.

Foraging decisions are made any time a bird has alternatives or options in terms of feeding locations or type of food (Tinbergen, 1981). When providing food for nestlings, adult

starlings are confronted with many options as to where they will forage and the type of prey items to be collected. Foraging decisions by adult starlings apparently involves a process in which nutritional value, caloric content, food abundance, effort and time required to collect prey, and brood demands are all factors in the selection of feeding locations (Royama, 1970; Tinbergen, 1981). Starlings will regulate their foraging activity according to their ability to maximize at least some of these factors. During the breeding season, starlings are central place foragers. Starlings will feed near nest sites if the local invertebrate population is sufficient and the aforementioned criteria are adequately fulfilled. However, starlings occupying nest boxes in a field in which the foraging is suboptimal may travel greater distances to forage in other areas. In order to reduce the influence that this will have on the ability to identify treatment effects, it is prudent to select paired field sites with similar adjacent surroundings. This is especially critical if the type of fields to be used for the study are not grasslands and do not appear to be optimal foraging locations for starlings.

Once a general field type (e.g., grassland) has been selected for use there are many other factors which should be considered. These include harvesting schedule of the crop, application of fertilizer to the crop, adjacent field types, proximity to competing nest sites, microclimate characteristics, edge habitat (i.e., woody non-agricultural areas), drainage characteristics, and presence of predators (especially raccoons). While it is often difficult to obtain detailed information on all of these parameters, interfield variability in reproductive success of starlings should be reduced through the selection of fields that have many similarities.

5.4.1.1 Hay Field Characteristics as an Example

The following discussion is provided as an example of the manner in which a grassland field site might be used for a pesticide field study. Although we provide justification for the use of hay fields as study sites, this is only applicable to pesticides that are registered for use on hay fields. Other field types are appropriate when a pesticide is not registered for use on hay fields.

Based upon information from the literature on starling foraging behavior during the breeding season, and from personal experience, we suggest that hay fields with at least one edge bordered by either deciduous, coniferous, or mixed deciduous/coniferous trees are preferred over hay fields without woody edge habitat. Woody edge habitat provides roosting sites and protection from predators for the starlings. The harvesting schedule of the grass also plays an important role in the use of a field by starlings. In general, the cutting and removal of grass from the field sites should take place according to the normal harvesting schedule. However, the grass should be cut and removed from the field within one week prior to the pesticide application. Cutting the grass at this time enhances the amount of time the starlings will spend foraging in the fields. As it is important to maximize the hazard potential from pesticide exposure, enhancing starling utilization of the study sites is strongly encouraged.

5.4.2 Previous Pesticide use in Study Sites

It is essential that a history of pesticide usage on selected field sites be documented and that adjacent areas do not have pesticides applied to them during the study period.

5.4.3 Geographical Location of Fields

During the breeding season starlings will seldom forage more than 200 m. from the nest site if food availability is adequate. However, starlings may fly up to 2 km from the nest if food sources are scarce near the nesting site (Feare, 1984). Observations of adult starlings during the first year of study should provide some information on typical travel distances used for foraging. These observations can be used to estimate the actual amount of time starlings spend in a study site and to determine if starlings are foraging in other study sites. In general, starlings will not travel more than one to two km from the nesting site. In order to reduce the possibility that starlings will leave their particular study site and foraging in another study area, it is important that study sites be located at least 3 km from one another.

5.4.4 Size of Study Area

The design suggested in this document is appropriate for agricultural fields that are 16.2 hectares (40 acres) is size. This size field is typically found throughout agricultural areas and is therefore a convenient size to use for a pesticide field study. Alternative field sizes can be used for a study. However, the design of a study using field sizes other than 16.2 hectares must also incorporate appropriate changes in the number of boxes used, the placement of boxes, and in the distance separating study sites.

5.5 Nest Box Design

Nest boxes should be constructed from utility grade lumber and should measure 29 cm. x 23 cm. x 38 cm. (l x w x h). The back portion of the nest box (the piece which attaches to the post)

should be 58 cm. in length. This piece should be centered so that 10 cm. extends above and below the nest box. These extensions serve as a surface through which the nest box can be mounted on the post. An entrance hole should be drilled in the front of each box 26 cm. from the base and centered on the facing board. The entrance hole should be 4.8 cm. in diameter. The top of each nest box should be attached by hinges and will serve as a door through which the nest contents can be observed. The hinged top should be latched by a hook-and-eye when closed.

The nest boxes should be attached to the top of 10 cm. x 10 cm. x 3.6 m. (4 in x 4 in x 12 ft) posts (although steel posts may be used as an alternative). A 50 cm. sleeve of aluminum sheet metal encircling the post should be placed approximately 15 cm. below the nest boxes. This metal sleeve will serve as a guard against predators.

5.6 Nest Box Placement

Thirty nest boxes should be placed in each field (assuming that a 16.2 hectare field is used). The boxes should be placed in a single row which bisects the field. The orientation of the row should be the same as that used for the operation of farm machinery (for the purpose of plowing, cutting, harvesting, etc.). The boxes should be separated from each other by 10 m. Nest box orientation (the direction toward which the entrance holes are oriented) may influence occupancy. Some suggestions that may increase the likelihood that boxes are occupied are 1) sequentially alternate box orientation with regard to the four cardinal directions (the orientation of the first box should be randomly chosen) or 2) face nest boxes away from predominant wind

direction. The posts should be buried approximately 60 cm. in depth so that the tops of the nest boxes are about 230 cm. above the ground. The posts should be firmly placed because an unstable post increases the likelihood of abandonment.

5.7 Date of Placement

Nest boxes should be placed well in advance of the breeding season for a given area. We recommend that the boxes be placed at least two months prior to the beginning of the breeding season. Specific dates for placement of the boxes can be derived from the literature on starling breeding biology within a specific area. Journals that are useful for obtaining information on the breeding biology of birds include: American Midland Naturalist, Ibis, Bird Banding, Auk, Wilson Bulletin, Journal of Field Ornithology, and the Condor. Information on starlings occupying specific areas can sometimes be obtained from persons associated with local chapters of the Audubon Society.

5.8 Duration of Study

5.8.1 For Evaluating Short Term Reproductive Effects of a Pesticide

Nest boxes on all fields should be monitored (refer to Section 5.9: Monitoring Schedule of Nest Boxes) from the time boxes are made available to the starlings until all birds occupying nest boxes on the day of pesticide application have had the opportunity to incubate their eggs and rear their nestlings. The specific time period implied by this designation is (assuming at least one of the eggs hatches): the number of days of observations during nest building, egg-laying, and incubation plus 33 days (12 days for incubation and hatching + 21 days for the

nestling(s) to fledge) following the date the last egg in the final clutch is laid (that is to say, the last clutch of eggs laid prior to the application of the pesticide). If none of the eggs hatch, but remain in the nest box, the study can be terminated when the last box occupied prior to the pesticide application has been monitored for a period of 12 days after the date of the laying of the final egg in the clutch.

If multiple applications of the pesticide are used, then the test can be terminated 33 days following the date of the laying of the last egg in the final clutch laid prior to the final pesticide application date. This is essentially the same criteria as described above, except that the date is adjusted for multiple pesticide applications; the final date of pesticide application is used as the date from which the termination of the experiment is defined.

5.8.2 For Evaluating Long Term Reproductive Effects of a Pesticide

If the compound being studied may cause prolonged sublethal effects on the adults and their ability to effectively reproduce, or if this possibility cannot be ruled out, then the field project must continue throughout the entire breeding season. The actual time indicated by this designation will vary depending upon the starling population utilizing the nest boxes. Starlings will typically produce two clutches of eggs and can sometimes produce three clutches. The principal investigator(s) will have to determine whether or not the breeding season in their particular area has been completed.

As a suggestion, investigators can reasonably assume that the breeding season is terminated for that particular site if 1) all eggs and nestlings from the second clutch have hatched and fledged, or died; and 2) at least two weeks has transpired without any eggs being laid in the nest boxes.

5.9 Monitoring Schedule of Nest Boxes

5.9.1 Frequency of Monitoring Nest Boxes

Once the nest boxes have been placed in a study site, an acclimation period of one week should transpire prior to the first day of monitoring. The boxes should then be monitored every fourth day throughout the nest site selection period. The boxes should be monitored every third day following the period of mate pairing. This period will be indicated by a dramatic change in the amount of material delivered to the nest and the care with which it is organized in the nest box. This change in nest building represents successful pair bonding and nest building activities being undertaken by the female and male together. Up to this point all boxes on a field will have been monitored on the same schedule. However, once nesting progress is different between boxes (i.e., observation of mate-pairing period) the boxes should be monitored on a per box basis rather than a per field basis.

The boxes should be checked every third day until an egg is observed in one of the nest boxes. Monitoring should then occur on alternate days until an egg hatches. Monitoring should take place daily while there are nestlings in the boxes.

5.9.2 Time of Monitoring Nest Boxes

It is important to minimize disturbances of the adult starlings. In order to monitor boxes with the least amount of interference to adult birds, it is advisable to monitor the nesting sites during the early afternoon hours. The hours between 11:30 and 15:00 are optimal for monitoring nest boxes. Monitoring at times earlier or later than this designated range results in a greater chance of disturbing an incubating adult or an adult involved in transporting food to the young. Under no circumstance

should nest boxes be checked prior to 10:00 a.m. during the egg laying period of the nesting season. Human disturbance during egg laying greatly increases the probability of nest abandonment by the female.

5.10 Data Gathered During Monitoring of the Nest Boxes

5.10.1 Reproductive Data

During each visit to a nest box the following information should be recorded:

Nest status: The presence of nest building material, initial phase of nest building, appearance of nest cup, completed nest (one egg observed in nest).

Number of eggs: The number of intact or broken eggs in each nest box.

Number of nestlings: The number of live and/or dead nestlings in each nest box. It is important not to handle the nestlings during monitoring. Although it is somewhat difficult to count all eggs and nestlings in a nest with more than four nestlings present, counts should be made only by observation. In the event that dead nestlings are found in the nest box, the number of dead nestlings should be recorded and the dead nestlings should be removed. All remaining live nestlings should be allowed to remain in the nest. Residue analysis and appropriate biochemical analyses should be performed on tissue taken from the carcasses.

A more detailed description of the information that should be recorded during the monitoring of nest boxes is contained in the support document under Section 2.8 (Reproductive Parameters).

5.10.2 Nestling Weight at 16 Days-of-age

All nestlings should be weighed at 16 days-of-age. Sixteen days-of-age is determined by designating the first day a nestling appears in the nest as day zero. Because all nestlings within a given nest box seldom hatch on the same day it is often difficult

to assign specific ages to individual nestlings within the nest box. In order to obtain reasonably accurate age designations it is important to weigh all nestlings on the date that the mean age of the nestlings is 16 days.

5.10.3 Other Information

Some examples of information that may prove useful to document, and that may enhance the ability to interpret the statistical analyses, are: presence of other species of birds in the nest boxes, presence of adults in nest, food substances observed in nest, and evidence of predators or predation (especially in the event of missing or dead nestlings or eggs). Additionally, food brought to the nestlings can be collected, by means of an esophageal ligature, and then used for pesticide residue analysis or to document the type of food brought to nestlings. While this information is useful, it may compromise the interpretation of reproductive data by influencing the survivability and growth rate of nestlings. Therefore, it should be done only if occupancy rates are sufficiently large so that additional boxes, in excess of the number needed for a statistically sound reproductive study, are availabe for use.

5.11 Data Sheet

Data sheets should contain the following information: date, investigators name, time of entry into and exit from the field, and general weather description. The data sheets must be designed so that all reproductive parameters described in Section 5.10 (Data Gathered During the Monitoring of Nest Boxes) can be clearly and easily documented. The data sheets should be collected daily by the principal investigator(s) or field project supervisor.

Duplicate copies should be made from each of the data sheets and stored at separate locations. The data sheets should be made of water resistant paper, and a permanent ink pen should be used for recording information on each data sheet.

5.12 Pesticide Application

5.12.1 Assignment of Pesticide Application to Fields

In order to prevent pseudoreplication the assignment of the pesticide application to a particular field must be randomized within each pair (Hurlbert, 1984).

5.12.2 Date of application of the pesticide

The pesticide should be applied according to label specifications. However, the actual date of pesticide application should be contingent upon the nesting schedule of the starlings. The pesticide should be applied to the fields when there are the greatest number of one to 14-day-old nestlings in the boxes. There will be some variability in the onset of egg-laying and hatching between and within fields. Fortunately, egg-laying and hatching among starlings occupying a similar geographical location are typically very synchronous (the onset egg-laying for most starlings occupying a given geographical location typically takes place within a four to ten day period) (Feare, 1984). This synchronicity in egg-laying and hatching can be influenced by disturbances, so it is essential that human intervention is minimized during the early stages of nest initiation and egg-laying.

5.12.3 Pesticide Concentration

This section specifically describes the methods required to investigate a liquid formulation pesticide. In the event that a granular pesticide is applied, the same principles descibed below also apply (e.g., calibration, verification of application rate, prior cleaning of tanks).

The pesticide should be applied at the highest accepted concentration for any crop. This concentration is used in order to simulate a 'worst-case' scenario for the pesticide. The application apparatus should be the same as that which typically would be used for the particular formulation being applied. The spraying apparatus must be calibrated prior to use. All holding tanks which will contain the pesticide must be thoroughly cleaned before the pesticide formulation is added. Samples from the final rinsing of the holding tank must be analyzed for the presence of residues, and the analysis must verify that the cleaning procedures have effectively removed any previous contaminants in the holding tanks.

The holding tanks should then be filled with the application solution and a minimum of six representative samples are to be taken to verify pesticide concentration. Of these six samples, three should be taken prior to the pesticide application, and three should be taken from the spray nozzles (or from a tank drain drain valve if present) following the pesticide application. The application rate must be verified by placing spray cards in randomly selected locations in the field just prior to the pesticide application. The number of spray cards necessary to document the application rate is dependent upon the type of spray card used and expected variability in the samples. The number to be used should be determined by the investigator. Immediately following the pesticide application the spray cards are to be collected. Each spray card must be placed into a storage

container that can be rinsed with hexane and acetone. All tank samples and spray cards should be promptly placed in a cooler with 'blue' ice and then transported to the laboratory for residue analysis. If the samples cannot be immediately analyzed, the pesticide should be extracted and then stored in an appropriate solvent at -20°C until analysis can be conducted.

5.12.4 Direction of Pesticide Application

Any pesticide that is aerially applied to the study sites should be applied in a direction perpendicular to the orientation of the nest box row. This is done in order to reduce the chance that the entire row falls on the swath overlap (area of higher pesticide concentration) of consecutive passes. If this cannot be accomplished (due to obstructions such as power lines or trees), then the pesticide application should be done in such a way as to ensure that only one swath lies directly over the boxes.

5.13 Statistical Analysis

An initial screening of reproductive data for differences between treatment groups should be conducted using a 2-way analysis of variance (randomized block design) with the number of fledglings per field as the reproductive parameter (dependent variable). It is advisable to transform this discrete variable, using the square root transformation, prior to analysis of the data sets (Sokal and Rohlf, 1981). If this initial test shows a significant difference between fields, then inferential statistics can be used to identify the specific parameter(s) of starling reproduction (at least those that were recorded during the study) that have been affected by the pesticide application. A significant difference in the number of fledglings per field

indicates that treatment effects occured if 1) the fields are blocked appropriately; 2) treatments are randomly assigned and interspersed; and 3) occupancy rates are similar across all fields.

The specific inferential and descriptive statistics to be used should be determined by the investigators. However, one caveat which should be considered is that many of the reproductive parameters to be compared and analyzed are described on a per nest basis rather than on a per field basis. Each field is the sampling unit and each box represents a subsample of the sample unit. It is quite easy to lose this distinction when using inferential statistics and to apply analysis of variance (ANOVA) procedures to data with the nest box being used as the sampling unit. Such procedures are invalid and utilize incorrect error terms and incorrect degrees of freedom (Hurlbert, 1984). Some reproductive parameters can be analyzed on a per nest basis if replicate values are available (e.g., weight of 16-day old nestlings). In such cases nested ANOVA procedures can be applied with the nest box being used as a subsample of the sample unit. The nested ANOVA procedure will maintain the proper degrees of freedom and a correct error term. Without replicate measurements per nest box, nested ANOVA procedures may not be appropriate (e.g., the number of fledglings per box) (Sokal and Rohlf, 1981).

A non-significant difference between treatment groups in the initial screening test does not mean that the pesticide did not have an adverse effect on starling reproduction (can be a consequence of the beta error or inappropriate selection of a variable). The number of fledglings per field encompasses nearly all aspects of starling reproduction as well as the occupancy of nest boxes. Because the number of fledglings per field is such a broad parameter, it is a useful indicator of gross differences between treatment groups, but it lacks sensitivity to minor differences. It is therefore important to also analyze other

reproductive parameters for significant differences. Pesticide exposure may cause a reduction in only one of the parameters measured (e.g., weight of nestlings) yet such a change is not detected in the initial screening test. Because of the potential for false negative results, conclusions regarding the hazard of pesticide usage can only be made after a very thorough analytical investigation of all parameters recorded during the nest box study.

TECHNICAL SUPPORT DOCUMENT FOR STARLING NEST BOX STUDIES

1.0 INTRODUCTION

1.1 Purpose

The purpose of this document is to provide background information and justification for the procedures and methodology described in the provisional nest box guidance document. This support document contains information derived from an extensive literature review of nest box studies, starling ecology, and toxicity testing. Also included in this document is information from a two-year field study that used nest boxes as a means of testing the effects of methyl parathion (an organophosphate insecticide) on starlings. The field study was used to test the efficacy of using starling nest boxes as a standardized method to examine the effects of pesticides on songbirds.

1.2 General Comment on Field Studies

Investigation of the effects of pesticides on birds has generally been limited to either laboratory tests of pen-reared species or to small-scale studies of captive birds. Although much useful information has been derived from these studies there exists a relative paucity of literature regarding the effects of pesticides on free-ranging birds under typical pesticide use conditions. The use of pen-reared or captive birds for toxicity testing has focused mainly on gallinaceous and/or waterfowl species (Subdivision E of the Pesticide Assessment Guidelines; EPA, 1982). The information on the toxicity of various compounds to these species has been extrapolated to other bird species. The extrapolation of toxicity data inherently introduces a

substantial amount of potential error. Extrapolation of toxicity data becomes increasingly difficult as differences between the species tested and those species which are being considered in a hazard or risk assessment increase. Substantial differences in feeding habits, distribution, breeding strategies, and habitat preference all contribute error to extrapolations of toxicity information between species of birds (Tucker and Leitzke, 1979). In order to reduce this error, it is necessary to expand the number of species tested and vary the conditions under which the testing occurs.

Large scale field studies are often plagued by a high degree of variability, uncertainty, and unaccountable intrusions (Hurlbert, 1984). Intrusions or sources of error enter into field studies at many levels; for example through differences in microclimate, species density and diversity, food availability, predation, and contamination. Sources of error can also be introduced through poor experimental design and execution. Thoughtfully designed studies which are carefully executed can help to eliminate unaccountable or nondetectable errors in a field study. Requirements for an effective field study include utilization of an appropriate number of replicates, randomization and interspersion of treatment groups (Hurlbert, 1984). Also necessary in an effective field study is standardization of methods used for the acquisition of data, which is limited in the the current field guidance document.

The standardization of methods employed in a field study assists the investigator in eliminating much of the ambiguity and uncertainty in the interpretation of data. Standardization of testing methodologies also allows investigators to compare data acquired from different experiments.

1.3 Nest Boxes:

Historically, nest boxes have been of great value in the study of reproduction in avian species. Boxes allow easy monitoring of nests and have been used to gain information on aspects of reproductive biology such as time of nest initiation, nest building activities, clutch size, frequency of egg-laying, egg viability, nestling survivability, fledging success, attentiveness of adults, and nestling food requirements. Many cavity-nesting birds have been studied using nest boxes, including American kestrels (Falco sparverius), barn owls (Tyto alba), wood ducks (Aix sponsa), European starlings (Sturnus vulgaris), purple martins (Progne subis), great crested flycatchers (Myiarchus crinitus), great tits (Parus major), flickers (Colaptes auratus), eastern bluebirds (Sialia sialis), tree swallows (Tachycineta bicolor), black-capped chickadees (Parus atricapillus), and house wrens (Troglodytes aedon) (Mitchell et al., 1953; Kessel, 1957; McGilvrey and Uhler, 1971; Dahlsten and Cooper, 1979; Greenwood et al., 1979; Marti et al., 1979; Fowler and Dimmick, 1983; Feare, 1984; Proctor, 1988).

Nest boxes have been used extensively for the study of the breeding biology and feeding ecology of starlings (Kessel, 1957; Feare, 1984; Tinbergen, 1981). Because starlings are tolerant of human activity and nest box alterations, these authors have been able to collect detailed information on the behavior and development of altricial birds. Starlings occupying nest boxes have been used by investigators to study the effects of pesticides on birds (Powell and Gray, 1980; Schafer et al., 1981; Grue et al., 1982; Grue and Hunter, 1984; Robinson et al., 1988; Kendall et al., 1988a; Stromborg et al., 1988). The information on bird responses to pesticides derived from these studies is indicative of the types of information gained from starling nest box studies.

Previous use of nest boxes, and in particular starling nest boxes, for obtaining data on the breeding biology, ecology, and response of starlings to pesticides strongly supports the decision to incorporate nest boxes into large-scale pesticide field studies.

2.0 SPECIFIC SUPPORTIVE INFORMATION FOR PROVISIONAL STARLING NEST BOX GUIDANCE DOCUMENT

2.1 Number of Fields

2.1.1 General Overview

A randomized block design is to be used for the starling nest box study. The blocks to be used for the study are pairs of fields; one treated with a pesticide and the other used as a control plot. Within this design, fields are matched according to physical similarities and each matched pair represents one block. This design reduces the number of fields that have to be used and therefore decreases the time and expense of conducting a nest box study (Fite et al., 1988).

The number of pairs of fields (blocks) to be used for a starling nest box study is determined from the reproductive parameter used as the initial screening test for differences between treatment groups. The number of fledglings per field is to be used as the primary parameter of starling reproduction to be documented and compared. Therefore, expected numbers of fledglings per field must be used to determine the number of pairs of fields necessary for a field test. The expected number of fledglings per field is then dependent upon occupancy of nest boxes, clutch size, and the fledgling to egg ratio. Values for these parameters were taken from Kessel (1957), Flux and Flux (1981), Feare (1984), Robinson et al. (1988), and Kendall et al.

(1988b). Listed below are values derived from each source and the derivation of the expected number of fledglings per field. The expected number of fledglings per field is then multiplied by a correction factor in order to provide a safety margin for studies in which occupancy and reproductive success are below expected values. The adjusted value is used in the determination of number of pairs of field sites necessary to detect a 20% reduction in the number of fledglings per field, where alpha=0.05 and beta=0.2. The equation is derived from the USEPA Terrestrial Field Studies Guidance Document (Fite et al., 1988).

2.1.2 Method

1) Occupancy:

Kendall et al.	81.5 %
Flux and Flux	78.5 %
Robinson et al.	77.5 %
Mean	79.2 %

2) Clutch Size:

Kessel	5.5
Feare	4.8
Kendall et al.	5.2
Mean	5.2

3) Fledgling/Egg Ratio:

Kessel	81.4
Kendall et al.	73.0
Feare	75.3
Mean	76.6

Using the mean values from these sources, the expected number of fledglings per field, for the first clutch only, and based upon thirty nest boxes per field =

(# of nest boxes)(occupancy)(clutch size)(fledgling/egg ratio)

(30)(0.792)(5.2)(0.766) = 94.60 fledglings/field

Adjustment for conservative estimate: (94.60)(0.5) = 47.30

The very conservative correction factor of 0.5 is applied because combined differences in occupancy, clutch size, and egg to fledging ratios may contribute to a reduction of up to 50% below expected values.

The number of pairs of fields can now be determined using the following equation:

$$n = (Z_{1-0.05} + Z_{1-0.2})^2 \quad \frac{4Q_1}{P_1^2 (1 + Q_1) c}$$

Where,

n = number of paired plots
$Z_{1-0.05}$ and $Z_{1-0.2}$ are critical Z scores
Q_1 = survival ratio
P_1 = mortality ratio
c = mean number of fledgling survivors on control plots

If alpha = 0.05 and beta = 0.2, and we want to be able to detect a 20 % difference in the number of fledglings per field, then we would require:

$$n = (1.65 + 0.84)^2 \quad \frac{4(.8)}{(.2)^2 (1 + .8) 47.30}$$

$$n = 5.83$$

Therefore, six pairs of fields should be adequate to fulfill the requirements of this test.

2.2 Field Site Selection

2.2.1 Field Characteristics

The fields chosen as study sites should all be of the same general type; that is to say the type of crop being grown on each field should be the same. Because the pesticide of study may be intended for use on a variety of crops, the investigator must decide which crop type to use for a starling nest box study. The

criteria for this selection process should include 1) crops which will have the highest application rate and frequency of pesticide application; 2) fields which are most frequently used by starlings for foraging; and 3) ease of acquiring a sufficient number of fields all within close proximity to each other. Fields should be geographically situated so that weather conditions (temperature, rainfall, and wind) will not differ substantially between fields.

Once the type of crop to be used for the study is determined, the field selection process will be based upon the following list of physical features of the fields. Each criterion is listed in bold and is followed by supportive information. It is the investigator's responsibility to prioritize the features of the various field sites to be used in the selection process and to select fields based upon an optimization of desirable features.

2.2.2 Starlings must use the study sites for foraging, roosting, or they must frequently fly over the study area.

Starlings must be observed in the study area prior to assigning a geographic area as a study site. Although this seems obvious, at least one starling nest box study has been attempted in an area in which previous starling usage had not been documented or confirmed. The investigator spent a considerable amount of time and expense placing nest boxes in their designated study plots to find that the starlings were present at very low densities. Starling occupancy of the nest boxes was extremely low, and the study was compromised.

Although starlings may not be observed to forage extensively or to roost within study sites, the frequency of flights of starlings over study sites may be sufficient to ensure that nest

boxes will be occupied. If starlings pass over study sites frequently and observe nest boxes during these flights, it is likely that they will preferentially select nest boxes over other nesting sites (Planck, 1967).

Starlings typically select nesting sites which are located within close proximity to foraging locations. Planck (1967) suggests that proximity to foraging locations and roosting sites are the most important factors in increasing the likelihood that nest boxes will be occupied by starlings. Because starlings forage for ground dwelling invertebrates (during the breeding season) in sparcely vegetated areas, optimal nesting sites are located near meadows, pastures, or grasslands. Information regarding starling breeding biology and foraging activities (Feare, 1984) and our experience indicate that pastures or hay fields are superior to other agricultural sites. Hay fields offer the investigator an advantage in that the amount of time the starlings spend foraging in the study site can be enhanced. This is most important in the days following the pesticide application and is accomplished by cutting and removing the grass shortly before the pesticide application. Starlings will forage almost exclusively in a hay field which has recently been cut, raked, and cleared (as long as the invertebrate population density and quality are sufficient to satisfy nutritional and caloric needs during the breeding season).

Use of hay fields for starling nest box studies enhances the amount of time starlings spend foraging in the field sites and facilitates the designation of replicate sites, since there is uniformity in the type and distribution of vegetation present in the field. Starlings probably select nest boxes as nest sites based upon availability of food sources, quality and availability of alternative nesting sites, competition for nest sites, and possibly the presence of proximal roosts.

2.2.3. The study sites as well as adjacent fields should be evaluated for pesticide residues or other toxic contaminants.

The study sites should be evaluated for any pesticides or other contaminants which may interfere with the goals of the study. A history of pesticide or other toxic chemical use in the area should be obtained. If there are any questions of pesticide residues remaining in the study sites, soil samples should be analyzed for the presence of contaminants. Residue analysis of soils from study sites should verify that the sites are free from pesticide contamination. Previous pesticide usage on adjacent fields should also be documented.

2.2.4 Pesticides should not be applied to adjacent fields during the study period.

Pesticides should not be applied to adjacent fields during the testing period. This is essential as starlings may forage in neighboring fields during a study, and if pesticides are being used in these fields this may affect the results of the nest box study on starlings. Interpretation of starling reproductive data would be difficult, if starlings were being concurrently exposed to other pesticides during the study.

2.2.5 Where possible, study sites should have at least some wood edge habitat.

Kendall et. al. (1988a) have found that the presence of some woody edge habitat enhances the likelihood that nest boxes will be occupied by starlings. Starlings are gregarious birds and communally roost in deciduous or coniferous trees (Feare, 1984). These communal roosts are large in the winter but are smaller during the spring and summer. During the breeding season available roosting sites are used by both male and female

starlings. Male starlings may select a nesting site and then attract a mate by singing or displaying from the nesting site or from nearby trees or other suitable roosting sites. Trees or large woody shrubs offer the starlings some protection from predators. When delivering food to the young, adult starlings will often fly from the foraging locations to a tree near the nesting site before flying to the nesting site (pers. obs.).

An adjacent woodlot with some starling nesting sites is advantageous in that it represents an area already occupied by starlings. An area adjacent to a study site that is attractive to starlings should improve the chance that starlings will observe and occupy nest boxes.

2.2.6 Adjacent fields should be used less frequently for foraging by starlings than the field being used for the study.

If adjacent sites are superior foraging areas, the frequency of foraging trips to the adjacent sites may be quite high. Foraging decisions among starlings are probably based upon the quality and quantity of food being gathered as well as the amount of energy required to collect the food samples. Starlings will return to foraging locations as long as the quality and quantity of food collected meets the needs of the nestlings (Tinbergen, 1981). If study sites are suboptimal foraging locations, but at least support an adequate invertebrate population, and if the adjacent sites are inferior to the study sites, then the starlings will probably spend most of their foraging time in the study sites. Conversely, if the study sites are suboptimal and adjacent sites support a large, diverse, and easily obtainable invertebrate population, then the starlings will spend most of their time foraging in the adjacent sites. If adjacent fields are superior foraging locations, the ability to detect pesticide related adverse effects (effects resulting from ingestion of

contaminated food or from dermal exposure to contaminated vegetation) on the starlings may be compromised due to the limited amount of time the starlings spend foraging in the treated field.

2.2.7 Study sites should be paired on the basis of physical characteristics.

During the first year of study the designation of field sites as pairs (treatment and control) is based upon similar physical features. The type of crop being grown on each of the fields within a given pair must be the same. Other features which must be considered when designating paired study sites include: harvesting schedule of the crop, application of fertilizer to the crop, adjacent habitat, other species of birds and mammals using the study sites, proximity to competing nest sites, microclimate characteristics, presence of predators e.g., raccoons (Procyon lotor), Cooper's hawks (Accipiter cooperii), sharp-shinned hawks (Accipiter striatus), great horned owls (Bubo virginianus), and feral cats (Felis sylvestris), soil invertebrate population, previous pesticide usage, and presence of other toxic substances. While there may be other factors that influence reproductive success, this list includes expected factors that may affect reproduction in starlings. Maximizing the number of similarities between paired field sites decreases the chance of having substantial differences in starling reproduction. Well matched pairs, with reduced heterogeneity in the variability of each reproductive parameter, will result in a decrease in the magnitude of the within blocks variability. Reducing this error term in the paired fields enhances the sensitivity of the experiment (Hurlbert, 1984).

2.2.8 Paired study sites must be separated.

Starlings typically forage within 200 meters of the nesting site, but may travel from one to two km if food availability is insufficient near the nesting site (Kessel, 1957; Tinbergen, 1981; Feare, 1984). When designating study sites as pairs (one treatment and one control) the two fields should be separated by a distance that will ensure that starlings occupying nest boxes on either of the paired fields will not forage in the other paired field. It is important that starlings occupying control fields are not exposed to pesticides. Information from studies (Feare, 1984) on foraging decisions during the breeding season indicates that three km in all directions is a sufficient minimal distance separating treated fields from control fields.

2.2.9. The planting, fertilizing, and harvesting schedule of the crop should be similar for all fields.

This criteria is most important for paired fields, but should be used for all fields. The planting, fertilizing, and harvesting schedule for fields should not differ by more than a few days. Farming activity can influence the amount of time starlings spend in a study site. This is particularly evident following crop harvest and disking or plowing of fields. In general, any activity that changes the abundance and accessibility of invertebrates will change the amount of time starlings spend foraging in a study site.

As an example, in a starling nest box study conducted in Ferndale, WA (Kendall et al., 1988a) the use of hay fields for foraging was increased immediately following the cutting and removal of the grass. Feare (1984) states that starlings will favor short grass over tall grass when foraging for invertebrates. An increase in the utilization of the study site for foraging by the starlings will increase the likelihood of

exposure to the pesticide via dermal and oral routes. The harvesting of other crops may cause a reduction in the frequency with which the starlings forage in a field. If harvesting of a crop decreases the availability of invertebrates, or if it causes a substantial disturbance to the starlings, the utilization of the study site for foraging may decrease.

If grass fields are used for a nest box study, the height of the grass at the time of pesticide application is of considerable concern. As stated above, starlings will prefer short grass fields to tall grass fields for foraging. It is therefore suggested to cut the grass prior to the pesticide application to enhance starling use of fields. It is also important that the grass be sufficiently tall at the time of pesticide application so that starlings come into contact with grass blades when foraging. Dermal contact to pesticides on vegetation can be an influential route of exposure in terms of the response of birds to pesticides.

2.3 NEST BOXES

2.3.1 Nest Box Design

The general nest box design described in the starling nest box guidance document has been used successfully in previous studies (Kessel, 1957; Robinson et al., 1988; Kendall et al., 1988b). Of these studies, the design described by Kendall et al. (1988b) is recommended for starling nest box studies. The justification for using this nest box design is threefold 1) the nest boxes are easily constructed; 2) the design conserves materials which reduces the cost of construction; and 3) Kendall et al. (1988b) reported very high occupancy of nest boxes.

As mentioned above, the primary reasons for using the design described by Kendall et al. (1988b) are the low cost and the ease of construction. The dimensions described by Kendall et al. (1988b) are similar to the original design used by Kessel (1957). However, Kessel's design was modified so that nest boxes could be easily constucted from utility grade dimensional lumber. The design minimizes the number of cuts necessary to construct the boxes by using the width of lumber (1 in x 10 in lumber is used) as the width of the nest boxes, therefore, only cross cuts are necessary. Effort, time, and expense of nest box construction are reduced by eliminating the need for longitudinal cuts. The nest box design by Kendall et al. (1988b) is also readily accepted by starlings as evidenced by the high nest box occupancy values reported by these investigators. During the second year of a two-year starling nest box study, they reported that 236 of 240 nest boxes contained completed nests. In this study four fields were used (only one of which was used in the previous year of study) and the percent occupancy of the boxes on each field were: 97%, 98%, 98%, and 100%. These are the highest nest box occupancy values of any studies reviewed, and because of these results it is apparent that starlings will use this type of nest box.

The dimensions of the nest box need not be precisely those suggested in the nest box guidance document. There are many possible designs that may work as well as, or better than, the design suggested. Alternative designs may be warranted if they are easier and less expensive to construct, and if they have been demonstrated to be used by starlings.

Perhaps the most important part of the nest box is the hole size. The diameter of the entrance hole will determine the species that occupies the nest box. If the hole is small (less than 1.5 inches) then species such as tree swallows (Tachycineta bicolor) or house wrens (Troglodytes aedon) may use the nest box

(Proctor, 1988). A large hole (greater than 3 in) may be used by kestrels (Falco sparverius) or flickers (Colaptes cafer) (DeHaven and Guarino, 1970). Fortunately, the starling is a very aggressive cavity-nesting passerine and usually is able to secure a nesting site in spite of competition from other cavity-nesting species. An entrance hole diameter of 4.8 cm (2 in) has been shown to be well suited to the starling (Kendall et al., 1988a and 1988b). This diameter allows easy access to the nest by the starling, yet is sufficiently small so that most larger birds or predators cannot access the nest box interior.

2.3.2 Nest Box Building Material

Wood is the building material of choice for starling nest boxes. Wood provides the nest with insulation from warm or cold environmental temperatures. Wooden boxes are readily accepted by starlings and are relatively inexpensive and easy to construct. Wood has been used extensively in starling breeding biology and toxicity studies (Kessel, 1957; DeHaven and Guarino, 1970; Tinbergen et al. 1981; Schafer et al., 1981; Grue et al., 1984; Robinson et al., 1988; Kendall et al., 1988a). None of these authors reported any problems associated with the use of wood as a nest building material.

Rough-cut lumber or lumber that has not been treated with preservatives and/or pesticides should be used for nest box construction. It is advisable to avoid the use of wood that has a high resin content or wood products that may release formaldehyde or other organic vapors.

It is important that the top of the box be hinged and then fastened to the box when in a closed position. If the top is not secured to the box when closed, winds can cause the box top to open. This may cause abandonment, or it may allow rain to saturate the nest. Undue exposure of the nest to rain through an

improperly fastened top could affect the survivability of the eggs or nestlings. A simple system such as a hook-and-eye works very well for fastening the box top to the box.

2.3.3 Predator Guard and Nest Box Height Above the Ground.

The predator guard as described in the nest box guidance document follows that used by Kendall et al. (1988a and 1988b). In their two year study Kendall et al. did not observe any evidence of predation of the nest boxes. Predators such as weasels (Mustela spp.), raccoons (Procyon lotor), and oppossum (Didelphis marsupialis) were known to inhabit the study sites but were probably prevented from climbing the posts and removing eggs or nestlings by the predator guard. In a field study in Iowa, all eggs, nestlings, and adults from 50 nest boxes (within one study site) were removed by raccoons on a single night (Larry Brewer, personal communication). In the Iowa study, PVC tubing was used as a predator guard. The tubing was placed around the post starting immediately below the nest box and extending down about 30 inches. It is possible that the raccoon was either able to grip the PVC tubing or was able to reach above the tubing and access the box. Because of the lack of predation reported for boxes which used aluminum sheet metal as a predator guard (Kendall et al., 1988a and 1988b), and the incidence of predation among those which used other methods, aluminum sheet metal should be used as a predator guard.

Aluminum sheet metal is typically available in rolls which are 50.8 cm wide (20 inches). This size aluminum sheet is satisfactory to use as a predator guard, however, larger widths are preferable.

The nest boxes should be attached to the top of 10cm x 10cm x 3.67 m (4in x 4in x 12ft) posts. The height is the same as that used by Robinson et al. (1988), and the dimension is the same as those used by Kendall et al. (1988b). This provides a very stout post and adequate height above ground to ensure that ground dwelling predators will not have access to the nest boxes. Other posts (e.g., steel posts) may be used as long as they provide a stable support for nest boxes and prevent predation.

2.4 NEST BOX PLACEMENT

2.4.1. General Overview

Placement of the nest boxes within study sites is very important as it will influence the occupancy of the nest boxes as well as the exposure of the birds to the pesticide. Objectives which must be considered when placing the nest boxes in the study sites are maximization of nest box occupancy and exposure to the pesticide, adequate separation between boxes to minimize aggressive interactions between neighboring nesting pairs, and effective recognition of each nest box by a pair of nesting starlings. If these objectives are adequately fulfilled, the ability to identify treatment effects should be greatly improved through sufficient sample size and exposure to the pesticide.

As mentioned previously in Section 2.2 (Field Site Selection), nest box occupancy may be enhanced by placing boxes in fields that do not already have a large number of available nesting locations for starlings. While this is a useful consideration when selecting the study site, it is not absolutely essential that the study site be located in an area completely free of potential nesting locations. It may be advantageous to select a field that has some existing nesting cavities. The presence of starling nesting sites indicates that starlings

already occupy the area and thus may enhance the utilization of nest boxes. Furthermore, if starling nest boxes are properly placed they may be preferentially used over other nesting sites. This is especially true if the the existing nesting sites are tightly clustered, poorly protected from wind and rain, and if there is much competition for the available sites. Therefore, appropriate placement of nest boxes so that they are easy to locate and recognize and are sufficiently separated from other nest boxes should result in a high percentage occupancy rate of the nest boxes.

2.4.2 Orientation of Nest Boxes

Orientation of nest boxes is an important factor that may influence occupancy rates and reproductive success. Verheyen (1980) found that nest boxes that faced south or east had the highest occupancy. The reason for this was not known, but it was inferred that these boxes were chosen because the interior of the nest box was warmed by the early morning sun. Kendall et al., (1988a) suggest that the orientation of nest boxes may be a factor in occupancy rates of nest boxes, and in the incidence of aggressive conflicts between starlings occupying neighboring nest boxes. In the first year of a two year study, Kendall et al. placed nest boxes (60 total per 40 acre field) in two parallel rows separated by a distance of 15 meters. The nest boxes were oriented so that the entrance holes of all nest boxes within a single row faced the same direction (away from the nest boxes in the other row). In the second year of their study (Kendall et al., 1988b) sequentially alternated the orientation of the boxes to face each of the cardinal directions (that is to say that the boxes were placed so that if the first box faced east the next would face north, then the third would face west, and so on until the row was completed). They placed 30 nest boxes in a single row centrally located within the study site and another row of 30 nest boxes along one margin of the study site.

In the first year of their study, Kendall et al. (1988a) reported that the percent occupancy for the four fields used were: 71%, 45%, 67%, and 70%. During the second year of study the percent occupancy of the four fields used were: 97%, 98%, 98%, and 100% (236 of 240 total nest boxes contained at least one clutch). Kendall et al. (1988b) also report that the number of conflicts between males establishing and defending nest sites was greatly reduced as was the number of disturbed or failed clutches in the second year of study. In the first year of study there were many instances of nest disturbance and egg removal. Boxes were observed with one or more eggs present, yet upon subsequent monitoring the eggs, and often the nesting material, would have been removed from the nest box. The investigators found numerous eggs intact or broken on the ground near the nests. Because intact eggs were found near boxes, and no evidence of predation was observed, it appeared that eggs had been removed by starlings other than the pair using the nest boxes. The incidence of egg removal, failed clutches, and aggressive interactions between starlings was greatly reduced in the second year of this study. The authors believe that the decrease in these factors in the second year of the study may be attributable to improved recognition of nesting sites by the starlings and/or to decreased clustering of nesting boxes. However, there are many other factors that may have contributed to occupancy and to the number of aggresive interactions between starlings. The inferences suggested are based on observations and were not tested.

2.4.3 Pattern of Nest Box Placement

Nest boxes should be placed in the fields so that planting, fertilizing, plowing, and harvesting of crops can be carried out with minimal hindrance. In order to achieve this, boxes should be

placed in rows which follow the orientation of the crops (the axis along which farm machinery will be driven). If 16.2 hectare (40 acre) fields are used, a single centrally located row is the suggested method for nest box placement.

The purpose of placing the row of boxes in the center of the field site is to provide a margin of treated area around the nest boxes. During the breeding season starlings will typically forage within 50 to 200 m. of a nesting site as long as a sufficient population of invertebrates are present (Kessel, 1957; Feare, 1984). Centrally placing the nest box row in a 16.2 hectare (40 acre field) leaves a distance of 201 meters from the center of the box row to the edge of the field and 56 meters from the row end to the field edge (boxes are separated by 10 m.). If an adequate food supply is available within the study site, this distance should be a sufficient margin so that starlings spend a majority of their foraging time on the study site.

The boxes should be placed 10 m. apart. This distance should provide adequate separation of boxes and a sufficient field margin surrounding the boxes. Kessel (1957) states that starlings will nest within close proximity (distances less than 10 m.) to one another without apparent errors in box recognition or increased aggression. Kessel further states that if the nest sites are very close together (less than 30 cm.) the nest sites will probably be occupied by only one nesting pair and many of the nest sites will remain unoccupied. Flux and Flux (1981) report that starlings will defend an area within one to two m. of the nest site. Based upon this observation Flux and Flux placed nest boxes two to three m. apart. In their study they had high occupancy rates (65% to 92%) over a six year study. However, Flux and Flux observed fighting among the starlings that were occupying the nest boxes and they reported mortalities and blinded starlings resulting from these aggressive interactions. Feare (1984) reports that a male starling with a mate will rarely

defend a nesting territory beyond ten meters. Robinson et al. (1988) placed starling nest boxes 10 m. from one another and had very high occupancy rates (73% and 82% on treatment and control fields respectively). Kendall et al. (1988b) also placed boxes at 10 m. intervals and showed high occupancy rates for the nest boxes. In the second year of study conducted by Kendall et al. (1988b) the authors observed minimal aggressive interactions between starlings which were occupying boxes located 10 m. apart. Ten m. separation between boxes is therefore suggested as a compromise between optimal separation of boxes and the need to provide a margin of treated area surrounding the boxes.

2.5 DATE OF PLACEMENT OF NEST BOXES

Nest boxes should be erected at least two months prior to the beginning of the breeding season for the area of study. Kessel (1957) and Feare (1984) report that starlings may investigate and roost in nest sites as early as October, but the most active time of nest site selection occurs during the months just prior to the breeding season. Kessel describes two winter populations of starlings: those that are communal roosters and those that roost in nesting sites. The starlings which roost in nesting sites are probably permanent residents of an area while the communal roosting population represents both permanent residents as well as those which are over-wintering in an area. The selection of nesting sites by the communal roosting starlings does not commence to any appreciable degree until two or three months prior to the breeding season, whereas the starlings which are nest site roosters may begin selecting nest sites in the late fall or early winter. While it may be useful to have nest sites available in the fall for selection by starlings, it is not imperative. Starlings which choose nest sites in the fall or winter continue to visit other nesting sites. The male starling will decrease the number of sites visited as the breeding season

approaches. The final site selected is usually chosen just prior to the breeding season (Kessel, 1957; Feare, 1984). Due to this behavior of continually searching for nest sites during the winter for both nest roosting and communal roosting starlings, the placement of nest boxes in study sites two months in advance of the breeding season should provide sufficient time for nest site selection by starlings.

In some areas erection of the nest boxes two months prior to the breeding season would result in an attempt to place the nest boxes in the study sites while the ground is still frozen. In such areas the climatic history of the area should be taken into consideration, and the boxes should either be placed in the late fall or early winter, or they should be placed immediately following the spring thaw. Investigators will have to determine whether or not they will be able to wait for favorable conditions to erect the nest boxes without substantial delay. As a general rule, if the nest boxes cannot be erected prior to one month before the breeding season due to late winter conditions, then the boxes should be erected in the late fall or early winter.

2.6 DURATION OF STUDY

2.6.1 Factors to be Considered

When determining the duration of the nest box study there are many factors which must be considered.

Mode of action
---What is the mode of action of the compound?
---Is the compound metabolically activated?
---What is the expected time lag between exposure and effect?
---Is the compound rapidly metabolized and excreted?

Environmental stability
---What is the expected rate of environmental degradation?
---How variable is the stability of the compound under various environmental conditions?
---What environmental conditions increase or decrease degradation of the compound.

Potential for bioaccumulation or biomagnification
---Does the compound bioaccumulate or biomagnify?
---If there isn't information on the compound, is its structure similar to other compounds which have been shown to biomagnify or bioaccumulate?
---Is the compound expected to be present in invertebrates?
---If the compound is expected to be found in soil invertebrates, what is the expected duration it will be present in these invertebrates?

Behavioral effects
---Does the compound produce prolonged behavioral aberrations?
---Is the compound similar to other compounds which have been shown to cause long-term behavioral aberrations?

Physiological effects
---Which physiological parameters of birds are expected to be adversely affected by the compound?
---Will the physiological effects be short-term or long-term?
---Which tissues will be adversely affected by the compound?

Population level effects
---Is there reason to believe that there will be long-term effects on population trends?
---Is one or two years sufficient to detect long-term population effects?
---Which endpoints will be used to determine population level effects?

2.6.2 General Overview

Once these factors have been considered and evaluated the duration of the study can be decided upon. There are two choices for study duration: short-term and long-term. The short-term study investigates the effect of the pesticide upon those starlings which are occupying the nest boxes at the time of pesticide application. The short-term study would not include information from clutches initiated after the application of the pesticide. Because of the timing of the pesticide application during the first brood (see Section 2.10: Pesticide Application), the short-term study is limited in the amount of information that will be derived. In the short-term study pesticide impacts on starlings are confined to those effects which occur directly or indirectly to the eggs, nestlings, or the adults during the

incubation of the first clutch and the rearing of the first brood. Prolonged or delayed effects on adult behavior, fecundity, egg fertility, egg shell quality, foraging ability, and attentiveness to the nestlings would not be observed. A short-term study is justified either as a screening study for a long-term study or if the pesticide being investigated as been thoroughly scrutinized in regards to the aforementioned criteria and there is no evidence which suggests that the pesticide will persist in the environment or cause prolonged physiological or behavioral changes in the birds. Because it is unlikely that a pesticide will meet these criteria most field tests will be long-term.

2.6.3 Short-term Study

The short-term study can be terminated 33 days after the laying of the last egg in the final clutch laid prior to the pesticide application. This assumes that some of the eggs in the final clutch have hatched. The final clutch is the clutch which was completed just prior to the application of the pesticide. The date of the laying of the last egg in the final clutch is the date after which no more eggs were laid in the nest. If the clutch was initiated prior to application of the pesticide and continues after the application date, then the date of the laying of the last egg is still used as the time from which the termination date of the study is to be determined. The figure of 33 days is based upon literature values for incubation duration and the typical amount of time necessary for nestlings to leave the nest. Collins and deVos (1966), Kessel (1957), Feare (1984), and Kendall et al. (1988a) generally agree that, for starlings, hatching occurs 12 days after the laying of the final egg in a clutch. These authors also showed fledging dates of 17 to 21 days following hatching. Therefore, it is reasonable to complete the short-term study, which only investigates the effect of the pesticide on starlings which had either eggs or nestlings in the

boxes on the date of the pesticide application, 33 days following the laying of the last egg in the final clutch laid prior to the date of the pesticide application.

2.6.4 Long-term Study

The long-term study is a much more thorough test. In the long-term test the starlings would be monitored throughout the entire breeding season. The long-term study would begin on the day the nest boxes are erected and would continue until all eggs and nestlings from the second clutch have hatched and fledged or died and at least two weeks have transpired without any eggs being laid in the nest boxes. The justification for using this criteria to terminate the long-term study is derived from information on starling breeding biology described in Dunnet (1955), Kessel (1957), Collins and deVos (1966), Dehaven and Guarino (1970), Feare (1984), Robinson et al. (1988), and Kendall et al. (1988a). Egg laying among starlings occupying a given geographic location is usually synchronous (especially with respect to the initiation of the first clutch); most clutches are started within four to ten days of each other. Following this initial peak in egg laying there is usually another peak in egg laying approximately 40 days later. There is also a smaller and less synchronous intermediate peak somewhere between the first and second clutch. Following the second clutch, egg laying drops off dramatically. There is seldom another clutch initiated beyond two weeks following the second clutch peak. There is widespread agreement that for most locations the initiation of egg laying is completed within two weeks following the second clutch peak. Based on this information the long-term study can be terminated if no new clutches have been initiated following two weeks after the peak date of the second clutch (the peak date of the second clutch is the mean date of all clutches initiated at least 35 days after the peak date of the first clutch).

2.7 MONITORING SCHEDULE OF NEST BOXES

2.7.1 General Overview

In a nest box study it is important to maximize the amount of information obtained, yet minimize the amount of disturbance to the nesting birds. This is accomplished by adjusting the frequency of monitoring nest boxes according to the commitment of the starlings to the nest site and to the type of information required. Monitoring of nest boxes during the early phases of the breeding season can be conducted infrequently (the birds are sensitive to human intervention, and the data on nest building need not be collected frequently). As the breeding season advances and eggs appear in the nest and begin to hatch, the monitoring must occur more frequently (it becomes increasingly important to obtain reproductive information on a frequent basis). Fortunately, with each phase of the breeding season the starlings become more committed to a nest site and are less likely to abandon if disturbed.

Researchers (Collins and deVos, 1966; Feare, 1984; Kendall et al., 1988a) have found starlings to be very tolerant of disturbance once nestlings are present in nests, but intolerant of excessive disturbance (such as handling adults at night) prior to hatching of the eggs. During the first year of a two-year study, Kendall et al. (1988a) monitored boxes every day throughout the breeding season and reported numerous instances of egg removal from nest and/or nest abandonment. During the second year of their study (Kendall et al., 1988b) these investigators checked boxes weekly, then every third day (during active nest building), every other day (when eggs were present), and then daily (when nestlings were present). During the second year of their study they did not report any unusual evidence of human disturbance to the starlings and concluded that their monitoring schedule was well tolerated by the starlings. Feare (1984)

reports that nest abandonment by starlings which have eggs in a nest will occur due to excessive disturbance by humans, especially if incubating birds are handled by the investigators. Based on this information, nest boxes should be monitored according to a schedule similar to that used by Kendall et al. (1988b).

2.7.2 Frequency of nest box monitoring

Following placement of the nest boxes in the study site, seven days should transpire prior to the first day of nest box observations. This allows time for the starlings to locate and adjust to the nest boxes without being disturbed by humans. During the period of nest site selection and initial nest building (by the male) nest box observations should take place every four days. Checking the boxes every fourth day will suffice to obtain the necessary information on the progress of nest building and site selection, yet it should not unduly disturb the starlings. Monitoring of nest boxes should be completed every four days until the breeding season advances beyond the nest selection period and enters into the mate pairing phase.

Nest boxes should be checked every third day following the time that the male has been accepted by a female and the pair have begun nest construction in ernest. This period is indicated by observing a distinct change in the manner in which the nest is constructed. As the breeding season progresses the amount of material in the nest box and the care with which it is placed in the nest box will change dramatically. Feare (1984) states that the male will establish a nesting location and will place nest building material in the nesting cavity. This behavior along with singing, wing flailing, bill-wiping, and occassional short flights are all used to attract a female to a nesting site and to promote pair-bonding. After a female has chosen a male and has

accepted the nesting location she will often remove existing nesting material from the nesting cavity and then begin building the nest with fresh material. The female constructs the nest in a noticeably thorough and definitive fashion; grasses, small twigs, leaves, feathers, and occasional man-made rubbish are carefully placed in the nest box. This is in contrast to the limited amount of material previously deposited in the nest box by the male and the disorganized manner in which the material was placed in the box. The male usually continues to contribute to the nest building process, but the bulk of the material brought to the nest and the organization of the nesting material is done by the female. The nest building process is quite rapid and within two to seven days the starlings usually have completed the construction of a well developed nest. Because of the observable difference in the quality of the nest and the amount of material introduced into the nest box following mate pairing, it is quite easy to know when to increase the frequency of monitoring nest boxes from every four days to every third day.

Once the nest is complete and environmental factors such as temperature and daylength are satisfactory, the female will begin laying eggs. The female will usually lay one egg every 24 hours until the clutch is complete (Kessel, 1957; Feare, 1984; Kendall et al., 1988a). The female will typically begin incubation following the laying of the last, or the penultimate egg in a clutch (Kessel, 1957; Feare, 1984). Because of the 24 hour period between the laying of each subsequent egg, the three-day interval in nest box monitoring allows investigators to monitor the nest boxes with sufficient frequency so that the laying of the first egg will probably not be overlooked by more than one day. Once eggs are observed in the nests the monitoring of the nest boxes should be done every other day. During this time period the starlings may abandon the nest if disturbed, yet it is important to monitor every other day so that the frequency of egg laying and the interval between the onset of

incubation and the date of the hatching of the first nestling can be determined with some degree of accuracy. Monitoring the boxes every other day is a compromise that minimizes disturbance to the starlings, yet information on the frequency of egg laying and the incubation period is still adequately documented.

Monitoring should be done every day once eggs begin to hatch and nestlings are present in nest boxes. The likelihood is very low of starlings abandoning the nest box once nestlings are present. Starlings have a substantial commitment to the nest at this point and are quite tolerant of disturbance by investigators (Feare, 1984; Kendall et al. 1988a). It is necessary to monitor the boxes every day while nestlings are present because it is important that the number of live and dead nestlings be accurately documented. It is also necessary to collect the dead nestlings for post mortem examination (e.g., residue analysis and appropriate biochemical analyses used to determine the probable cause of death). If the boxes are not checked each day the likelihood of collecting dead nestlings decreases; adult starlings will often remove dead nestlings from the nest. In the event that adults remove dead nestlings from the nest, it is improbable that investigators would locate the dead nestlings in the field. Also, many of the biochemical analyses (e.g., cholinesterase activity) that are used to determine cause of death, or to attribute death to pesticide exposure, require that the carcass be collected shortly after death (Ludke et al., 1972; Hill and Fleming, 1982). Decomposition of a carcass can adversely affect the ability to effectively utilize biochemical assays for the determination of toxicant-induced mortality.

2.7.3 Time of Monitoring Nest Boxes

Starling activity about the nest boxes is greatest during the morning, late afternoon and evening hours. This is true during nest building, egg-laying, incubation and during the rearing of the nestlings (Dunnett, 1955; Kessel, 1957; Tinbergen, 1981; Feare, 1984; Kendall et al., 1988a). Most authors agree that the morning peak in activity drops off around 10:00 and resumes again in the late afternoon or early evening. It is during the mid-day window of time (11:30 to 15:00) that monitoring of the nest boxes should be undertaken. By monitoring during this time of day disturbances to the starlings will be minimized, and therefore the chance of causing the starlings to abandon the nest box will be reduced.

2.8 REPRODUCTIVE PARAMETERS

2.8.1 General Overview

The following is a list of reproductive information that should be recorded, or calculated from nest box observations, in a starling nest box study. This list represents the minimal amount of information necessary to adequately document any reproductive perturbation(s) that occur(s) as a result of pesticide exposure to starlings using nest boxes.

1) Date of nest box selection (based on the presence of some nesting material).

2) Notes on the development of the nest (amount of nest building material, quality of the nest, presence of a nest cup, etc.).

3) Date of the laying of the first egg (the previous day is designated the date of nest completion).

4) Interval of egg-laying (should be about 24 hours).

5) Clutch size.

6) Number of missing eggs or broken eggs.

7) Number of eggs that hatch.

8) Date of egg hatching.

9) Number of missing or dead nestlings.

10) Weight of 16-day-old nestlings.

11) Number of fledglings.

12) Date of fledging.

Note: The definitions for these parameters are described in Section 2.0 (Definitions) of the nest box guidance document.

Any changes in starling reproductive success should be made apparent by documenting and interpreting these parameters of starling reproduction. Differences in the reproductive success of starlings between or within treatment groups can then be thoroughly investigated by means of identifying which aspects of starling reproduction were adversely affected. Identifying the parameters of reproduction that are significantly altered by exposure to the pesticide is very useful for investigators, regulatory agencies, and pesticide producers. Specific information on the parameters of reproduction that are affected by a pesticide can be used to determine if reproductive perturbations might be reduced through changes in the usage pattern or formulation of the pesticide. This information will also assist investigators in identifying those parameters that should receive close scrutiny in future pesticide studies.

The aforementioned list of starling reproductive parameters is not an all-inclusive list of potential reproductive parameters that can be recorded by researchers. It is the minimal amount of information necessary to conduct a reasonable reproductive study of a local population of starlings occupying nest boxes. Certainly there are many other parameters which could be investigated in a nest box study. Additional information that

can be obtained includes: measurement of enzyme activity in the blood or other tissues (e.g., blood and brain cholinesterase activity), residue analysis of various tissues, tissue damage, behavioral changes, and post-fledging survival rates. The type of information gathered in a nest box study is dependent upon the specific questions being addressed and the hypotheses being tested.

2.8.2 Weight of Sixteen-Day-old Nestlings

The weight of nestlings should be recorded on the 16th day after hatching. The determination of weight of nestlings is necessary as it is an indicator of the growth rate of the nestlings. The growth rate of the nestlings is dependent upon the amount and quality of food brought to them, their health (e.g., begging behavior, metabolism, nutritional status). and the attentiveness of the adults. Nestling growth and weight gain is also an indicator of the general fitness of nestlings, their resistance to environmental stress, and the likelihood of surviving post-fledging. Recording the weight of 16-day-old nestlings allows the investigator a means of identifying potential sub-lethal effects of pesticide exposure (weight loss, slow growth, reduced vigor) that would have remained undetected if only nestling survival and fledging success were recorded. This is important as pesticide (organophosphate pesticide) exposure can cause a reduction in nestling weight either through direct effects (e.g., reduced begging behavior) or indirect effects (e.g., decreased food availability). Exposure to organophosphate insecticides has been shown to reduce the attentiveness of adults (Grue et al., 1982), to impair the begging behavior of nestlings (Grue and Shipley, 1983), and is associated with reduced weight of nestlings at fledging (Powell and Grey, 1980). Any of these factors could cause profound effects on the well-being of the nestlings and on the likelihood that fledglings would survive post-fledging.

The weight of the nestlings just prior to fledging may be a useful indicator of the likelihood that they will survive post-fledging (Crossner, 1977; Stromborg et al., 1988). The post-fledging period represents perhaps the most critical time period for a young songbird. A large percentage of fledglings (56% to 76%) die during the first few months post-fledging (Feare, 1984; Coulson, 1960). The ability of fledglings to survive during the first few months post-fledging is dependent upon their health status upon fledging, their ability to learn to forage efficiently and effectively, their ability to escape from predators, environmental conditions, and the attentiveness of the adults. Fledglings which are of low weight, weak, or otherwise impaired prior to fledging have a reduced chance of surviving during the months post-fledging. The weight of the nestlings at the time of fledging is therefore an important factor determining post-fledging survivability.

We have recommended that the nestlings be weighed at 16-days-of-age for a variety of reasons. From studies on the growth and development of starling nestlings it has been shown that the weight of nestlings increases rapidly during the first two weeks of incubation and then stabilizes following the 15th day (Feare, 1984; Kessel, 1957; Dunnet, 1955). These authors have measured the weight of nestlings throughout their development and all papers reviewed showed a plateau in nestling weight on the 15th and 16th days of age. Because the weight of nestlings stabilizes by the 16th day-of-age, it is a reliable indicator of the weight of the nestlings prior to fledging. The weight is also to be recorded on the 16th day-of-age because handling of the nestlings later may cause premature fledging of the nestlings from the nest box (Feare, 1984).

As mentioned above, the nestling weight stabilizes around the 15th or 16th day-of-age. However, there is some indication that the weight may decline slightly just prior to fledging.

Weighing the birds just prior to fledging (around the 20th day-of-age) would give a more accurate estimate of fledgling weight (nestling weight immediately prior to fledging), however this may influence the day that the nestlings leave the nest. It is important not to alter the fledging date because differences in age at fledging between and within treatment groups provides valuable information on nestling behavior and fitness. Fledging age which is consistent between treatment groups or agrees with values cited in the literature on 'normal' starling populations suggests that behavioral, physiological, or environmental conditions (such as exposure to a pesticide) probably have not caused a profound deviation in the behavior of the nestlings (at least in terms of their ability to respond to cues involved in the decision to leave the nest). Information such as the age at fledging provides investigators with data that can be used to evaluate subtle sub-lethal effects (e.g., behavioral changes) of pesticide exposure.

2.9 DATA SHEETS

Information gathered during visits to nest boxes must be accurate, clearly written, and recorded in a manner which minimizes error. Properly designed data sheets can greatly facilitate the recording of data. It is important that the data sheets used in nest box studies are easy to read, allow sufficient room for each entry, have a specific location for each entry, are printed on water resistant paper, and facilitate the ease of transcribing data from data sheets to a computer data base. Entries on the data sheet must be recorded in permanent, waterproof, black ink. As it is likely that observations of nest boxes will occur during inclement weather, recording of information on data sheets must be done in a manner which will not be destroyed or rendered unusable by moisture.

Information to be recorded on a data sheet should include:

1) Observer's name.

2) Date

3) Time of entry and exit from the study site.

4) Location (study site)

5) Nest box number.

6) A place for all reproductive information listed in Section 5.10 (Data Gathered During Monitoring of the Nest Boxes) of the starling nest box guidance document.

7) Weather information (max. and min. temperature, precipitation, wind speed).

8) Comments or observations. Often comments by observers can be useful in the interpretation of data. Comments indicating the presence of damaged nest boxes, presence of predators in study sites, interesting starling behavior, etc. should be included during the daily observations of the nest boxes.

Data sheets should be photocopied and originals should be stored in an appropriate location (e.g., fireproof file cabinet). The photocopies can then be used for transcribing data into a data base or for use in a statistical data file. All data sheets and data files should be checked for accuracy.

2.10 PESTICIDE APPLICATION

2.10.1 Pesticide Concentration, Frequency of Application, and Number of Applications.

The pesticide concentration to be applied to study sites will be determined from the proposed use of the pesticide. The pesticide concentration should be the highest proposed concentration for use on the type of crop present in the study

area. The justification for using the highest concentration is that it is necessary to simulate a 'worst-case' scenario for the pesticide being tested. This is consistent with the guidelines set forth in the Field Guidance Document (Fite et al. 1988). The number and frequency of pesticide applications to be used during the starling nest box study should also be determined from the proposed use of the pesticide. The number of pesticide applications and the frequency of the applications should be consistent with the highest proposed rates on the pesticide label. The rational for this is the same as that used for the concentration of the pesticide: it is essential that the hazard potential to starlings from the pesticide be maximized.

2.10.2 Date of Initial Pesticide Application

The timing of the initial pesticide application should be dependent upon the age class of starling nestlings present in the nest boxes. The initial application of the pesticide, or the only application of the pesticide in the event that a single application is used for the starling nest box study, should be applied to the fields when there is the greatest number of one to 14-day-old nestlings in the nest boxes. This date is chosen as it increases the likelihood of the nestlings being exposed to the pesticide via dermal absorption, ingestion of contaminated food substances, and direct inhalation on the day of spraying (if the pesticide is aerially applied). Increasing the number of routes of exposure to the pesticide (when residue levels are the highest) improves the likelihood of observing pesticide induced effects on starlings.

2.10.3 Exposure Routes and Nestling Sensitivity to Pesticides

Dermal exposure of adult starlings to a pesticide should occur as the adults forage in study sites and pesticide residues adhere to their feathers, legs and feet. The adults may absorb these residues (Wilson and Hooper, 1988) and/or they may transport pesticide residues to nestlings. Contamination of nestlings via dermal exposure is most likely to occur while adults are brooding nestlings. Adult starlings will brood nestlings during the first few days following hatching and will continue to brood the young for seven or eight days post-hatching (Kessel, 1957; Feare, 1984). During this time period the nestlings have little, if any, dermal protection from feathers. Nestlings start showing signs of feather tracts on the third or fourth day, and around the sixth day feather shafts begin to protrude through the skin. From the seventh day onward, feather tips begin to emerge from the feather shafts and some downy feathers also begin to appear. Feather shafts will continue to grow until nestlings are around 14 days of age. At this time the feather shafts begin to split and feathers emerge from the feather shaft. At this stage feathers cover most of the nestling's body surface providing insulation and protection for the nestling. The potential for absorption of a pesticide via the dermal route will be greatest if the pesticide is applied while the nestlings are less than two weeks old.

Another reason for applying the pesticide to the study sites when there are the greatest number of young nestlings (one to 14-day-old) present is to increase the likelihood of exposure to the pesticide via ingestion. Throughout the nestling rearing period the adult starlings will feed the young. The frequency of sorties from the nest increases during the first ten days and then levels off until around the 15th day, after which it declines somewhat (Feare, 1984). The frequency of the visits to a nest box and the amount of food delivered to nestlings is

dependent upon the number of nestlings present, the hunger of the chicks, and the availability of food (Tinbergen, 1981). Feare (1984) reports that the number of sorties from a nest box (after the 10th day) required to satisfy the nutritional demands of nestlings can be as many as 300 per day! During this time the adults will forage close to the nest box if food supplies and the nutritional quality of food samples are adequate (Tinbergen, 1981). The likelihood of pesticide exposure to the nestlings via ingestion is enhanced by maximizing the pesticide concentration and the number of days the pesticide is present in the study area during the nestling rearing period. This is accomplished by spraying the pesticide while the maximum number of young nestlings are present in the nest boxes.

The application of the pesticide to study sites while there are the greatest number of young nestlings present increases the likelihood of showing an adverse response to the pesticide due to the physiological and biochemical condition of young nestlings. The ability of nestlings to survive acute or chronic insult from pesticides is dependent upon the efficiency and rapidity with which the compounds are metabolized and excreted. Age-related differences in susceptibility results from lower activity in enzymes which detoxify or conjugate xenobiotics. The buffering capacity of the blood and the development of the blood-brain barrier may also contribute to age-related sensitivity to xenobiotic exposure (Doull et al., 1980; Goodman and Gilman, 1985). Age-related sensitivity in starlings has been reported in the literature. It has been demonstrated that young nestlings were more sensitive to an organophosphate pesticide than older nestlings (Grue and Shipley, 1983). In this study, the investigators reported that brain cholinesterase and body weights were reduced more in five-day-old nestlings than in 15-day-old nestlings following intubation with dicrotophos. These authors also found that the 24-hr median lethal dose of dicrotophos (phosphoric acid 3-(dimethylamino)-1-methyl-3-oxo-1-propenyl

dimethyl ester) for five-day-old nestlings was about one-half that demonstrated for 15-day-old nestlings. Hudson et al. (1972) found that the LC_{50} values for eight out of ten anti-cholinesterase compounds tested showed increasing values between age groups of mallards aged 36 hr, seven day, and 30 days of age, yet they showed a decreasing trend of LC50 values for birds aged seven or 30 days and birds that were six months-of-age. The information from these studies and others (Wolfe, 1988) suggests that young nestlings, or ducklings, may be more susceptible to pesticide exposure than are older nestlings, or ducklings, and adults.

In summary, the application of the pesticide to the fields when there is the greatest number of one to 14-day-old nestlings should maximize the potential for pesticide exposure by a variety of routes. The likelihood of observing a response to pesticide exposure is enhanced by applying the pesticide at a time when the nestlings are the most sensitive to toxicants.

2.10.4 Verification of Pesticide Application

The pesticide mixture applied to study sites should be verified by chemical analysis. Verification can be accomplished by sampling the holding tanks (assuming that a liquid formulation is used) prior to and following application of the pesticide. If the pesticide to be applied is a granular formulation, it is imperative that the granules be evaluated for the concentration of active ingredient. The number of samples taken depends upon the inherent variability in sampling and residue analysis. It is the responsibility of the investigator to determine the appropriate number of samples to be used for analysis.

Field verification of the application must also be accomplished. Granular application rates can be verified by measuring the deposition rate of the granules from the dispensing

apparatus. Spray cards can be used to verify the application rate of liquid formulation pesticides. The number of cards to be used depends upon the inherent variability in the application system and the variability attributable to sample card analysis. Values describing variability may be obtained from previous field tests using spray cards. If this information is unavailable then it may be necessary to conduct a preliminary experiment to determine the variability in documenting a pesticide application. Once the variability has been established, the proper number of cards can be determined using the following formula (Sharaf et al., 1986):

$$n = \frac{Z^2\sigma^2}{\epsilon^2}$$

Where;
Z=the standard normal variable (tabulated)
σ=the population variance
ϵ=the allowable variance

Residue data from granular pesticide applications on 16.2 hectare (40 acres) sites has indicated the need for 56 samples (Cobb et al., 1989). This number would provide a 90% probability that the mean concentration had a relative standard deviation (RSD) of $\pm$ 10%. Aerial applications on 16.2 hectare fields provide data which suggest the need for 51 samples to achieve a RSD of $\pm$ 10% (Kendall et al., 1988b). These sample numbers can be lowered by analyzing the samples immediately (within 24 hours) following the spray application, or by extracting pesticide residues and storing samples at -20^{o}C.

Spray cards should be placed randomly on the field of interest. This placement can be accomplished by establishing a coordinate grid over the entire field and randomly choosing a coordinate pair for the sampling point (Cobb et al., 1989). The number of coordinate pairs should be 50% greater than the number

of samples to be taken. This will allow points falling in unusable locations (e.g., wooded areas or drainage ditches) to be excluded from the sampling scheme. Once the application is complete, spray cards should be promptly collected. Each sample should be placed in a container that will not disintegrate, or release interfering compounds, when rinsed with hexane or acetone. The sample containers should then be placed on 'blue' ice until they can be transported to the lab for chemical residue extraction and analysis.

2.11 STATISTICAL ANALYSIS

2.11.1 General Overview

The guidance document states that reproductive success should be initially analyzed using a 2-way ANOVA randomized block design, with the number of fledglings per field as the dependent variable. This initial test is suggested as a broad screening for potential treatment effects. However, final interpretation of pesticide effects on starlings cannot be limited to this test. Further statistical analyses of other reproductive parameters must be undertaken in order to either accept or refute the hypotheses being tested. Reproductive parameters (e.g., the mean number of fledglings per box per field, mean clutch size per box per field, mean number of nestlings per box per field, mean weight of 16-day-old nestlings per field, etc.) all must be considered prior to reaching a final judgement regarding treatment effects.

It is beyond the scope and intention of the guidance document and this support document to expound upon possible methods for the analysis and interpretation of data. The actual statistical analysis of data sets from a starling nest box study will probably exceed (in detail and content) the tests suggested

in the guidance document. The use of appropriate statistical procedures for interpretation of data are the responsibility of the investigators and should be tailored to the questions being addressed in each field test.

2.11.2 Initial Screening Test of Reproductive Success

The starling nest box guidance document suggests that the primary measure of starling reproduction is the number of fledglings per field. This parameter is suggested because ultimately it is the number of nestlings which survive and fledge that determines the number of starlings introduced into the wild population. This parameter is defined in terms of the experimental unit for the study (the study site). The number of fledglings per field is used in the comparison between study sites as it is the integration of all aspects of starling reproduction (i.e., nest box occupancy rate x eggs per nest x percent hatchability x percent fledging). The number of fledglings per field is therefore, a parameter which incorporates variability resulting from differences in starling reproductive success, pesticide effects, and factors attributable to the specific study site.

Of primary concern in the starling nest box study is the ability to identify treatment effects (pesticide induced changes in reproductive success). In order to effectively identify pesticide induced reproductive perturbations it is necessary to sort out differences due to other factors such as occupancy rates (if boxes are occupied prior to the pesticide application) and field effects. Field effects are those factors associated with the study site, but are not due to treatment effects. Factors pertaining to a specific study site that may influence the degree of reproductive success include differences in the availability and quality of food, microclimate conditions, predation, and competition by other birds for nesting sites or food. Nest box

occupancy may also differ between the study sites. Occupancy of nest boxes has a large influence on the number of fledglings per field and must be accounted for in the evaluation of differences between treatment groups. Differences in reproductive success that are attributable to occupancy or field effects should be identified and analyzed for significance prior to attributing reproductive perturbations to treatment effects.

Careful interpretation of the data will allow the investigator to either refute or accept the hypothesis being tested. Differences in the number of fledglings per field can only be attributed to treatment effects if field effects are insignificant and inherent variability is sufficiently low. The ability to reduce differences in reproductive parameters among treatment groups (that is to reduce the variability among treatment groups) is accomplished in large part by careful selection of study sites and through the design of the field study. A study design which utilizes a sufficient number of replicates and has adequate interspersion of treatments among replicates should help to reduce the variability between fields.

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APPENDIX 1

SELECTION OF THE STARLING AS A TEST SPECIES

Introduction

There is increasing concern about the effectiveness of pesticide testing procedures to accurately predict hazard to wildlife species. The current process involves using laboratory tests, reproductive studies, mesocosm tests, and field studies to assess the potential hazard of pesticide usage to non-target organisms. Unfortunately the number of species currently used is very low (often the number of mammal or bird species used for toxicity testing is just two species) and the inherent error in extrapolating toxicity results to other wildlife species may be substantial. Error in extrapolating toxicity data between wildlife species can be introduced through differences in foraging behavior, food preference, geographical distribution, size and weight, metabolism (basal metabolic rate, metabolic enzyme activity, excretory processes, etc), environmental conditions, age, aversion or avoidance response to toxicant exposure, route of exposure, among others. In order to reduce the error in extrapolating data between species it is necessary to increase the number of orders and families of wildlife represented in toxicity testing.

Wildlife Species for Toxicity Determinations

Of the 650 species of birds found in North America, pesticide testing has been primarily limited to just two species; the northern bobwhite (Colinus virginianus) and the mallard (Anas platyrhynchos). The mallard is of the Order Anseriformes and the northern bobwhite represents the order Galliformes. These two Orders of game birds represent around five percent of the total avifauna in North America (Schafer and Brunton, 1979). Passeriformes is the largest order of birds (the perching birds). The Order Passeriformes contains 23 families (Robbins et al., 1983) and constitutes about 50 percent of the total population of birds in North America (Schafer and Brunton, 1979) and more than half the world's population of birds (Farrand, 1983). Within Passeriformes some of the larger families are the Sturnidae (starlings), Icteridae (blackbirds and orioles), Passeridae (old world sparrows), and Fringillidae (new world sparrows, grosbeaks, finches, and longspurs) (Schafer and Brunton, 1979). Because of the population size and the percentage of all birds represented by Passerines it is important that pesticide testing procedures include species from this Order.

Criteria for the Selection of Test Species

Factors which should be considered when determining an appropriate species of bird to use for toxicity testing include: geographical distribution, population size, feeding behavior, potential for exposure to pesticides, pharmacological susceptibility to pesticides, economic and aesthetic importance to man, feasibility of using the species (tolerance to handling, pen size requirements, survivability in captivity), reproductive rate, existing data base (ecology, toxicity data, breeding biology), susceptibility to disease, permit requirements, and similarity to other species (phenotypic, genotypic, behavioral, diet). It is unlikely that any single species will satisfy all of these criteria. However, by using a variety of species, which satisfy at least some of these criteria, the ability to extrapolate toxicity data to other birds will be improved.

Of the Passerines, the Sturnidae, Icteridae, Passeridae, and the Fringillidae are perhaps the most amenable to toxicity testing. Species from these orders occur in large numbers, are easily captured, can be housed in indoor or outdoor facilities, and are tolerant of handling. Some species such as starlings (Sturnus vulgaris), house finches (Carpodacus mexicanus), red-winged blackbirds (Agelaius phoeniceus), and house sparrows (Passer domesticus) are economically important to man. In agricultural areas of North America extensive crop damage has been reported by these birds (Feare, 1984; Farrand, 1983; Welty, 1982). In contrast, these same species, which have been designated pest species, have also been introduced into agricultural areas to control insect pests (Welty, 1982; Farrand, 1983).

Two very distinct considerations prior to using a bird species for toxicity testing include the need for permits and potential opposition. Federal permits are required for the collection and captivity of most species of birds. However, house sparrows and starlings can be captured and used for toxicity testing without obtaining a federal permit (state permits may be required). These species are considered pests and in comparison to other species of birds there is minimal opposition to their use in pesticide toxicity tests.

In terms of large-scale pesticide field studies, species that are cavity-nesting birds (starlings, house sparrows, house finches, tree swallows (Tachycineta bicolor) are particularly amenable to toxicity testing. These species are often found in or near agricultural areas, and local populations can be encouraged to nest in specific study sites through the introduction of artificial nest boxes into an area. The likelihood that these birds will occupy nest boxes is dependent upon competing nest sites, availability of food resources, nest

box design, density of local bird populations, and the tolerance of each species to the presence of man. The starling has been shown to consistently and readily occupy nest boxes whereas other species are not as likely to occupy nest boxes (Kessel, 1957; Kendall et al., 1988).

The likelihood that a test species will be adversely affected by a pesticide is dependent upon exposure and pharmacological susceptibility. Exposure to a pesticide can occur by inhalation, dermal absorption, and ingestion. Nesting location and foraging activities are the primary factors determining the degree of exposure to a pesticide during the breeding season. Birds which nest in areas which receive a direct application of a pesticide are more likely to be exposed to a pesticide (which is applied during the breeding season) than are those species that nest outside of an application area. The potential for exposure to a pesticide is greatly influenced by the degree of contamination present on food samples consumed by birds. In order to increase the potential for observing pesticide-induced perturbations in a test species, it is necessary to select a species that has the greatest potential for exposure.

Of the species which are suitable to nest box studies the starling, house sparrow and house finch have the greatest potential for exposure to an agricultural pesticide. During the breeding season the starling will forage for ground dwelling invertebrates (Feare, 1984). By foraging for ground dwelling invertebrates the starling increases its chances of exposure to pesticides through dermal absorption and through ingestion of contaminated insects. The house sparrow and the house finch are primarily vegetarians, although they will consume invertebrates during the breeding season, (Proctor, 1988; Farrand, 1983). These birds may be exposed to pesticides through the consumption of contaminated vegetation and via dermal exposure. The starling should have the highest exposure to pesticides that accumulate in the tissue of invertebrates and/or are found in high concentrations in the upper centimeters of the soil (i.e., granular pesticides).

In terms of the pharmacological susceptibility of Passerines to toxins, it appears that the starling may be somewhat more resistant than other species. Schafer (1972) has reported that the starling is consistently more resistant than is the red-winged blackbird and is probably more resistant than the house sparrow (Schafer and Brunton, 1979). The house sparrow, house finch, and red-winged blackbird do not show definitive differences in susceptibility to a variety of pesticides. These studies and others (Schafer and Cunningham, 1972) suggest that toxicity data from adult starlings may not be indicative of other passerines. Recent research by Wolfe (1988) has revealed that nestling starlings (3-day-old) can be up to 20 times more

sensitive to diazinon than adult starlings. It is apparent from these studies that extrapolation of toxicity data from starlings to other species of birds must be carefully considered.

Definitive evidence that starlings are more resistant to pesticides than other birds is lacking. The apparent resistance of starlings to pesticides is based upon a limited number of endpoints and upon extrapolation between studies. Most studies have used mortality as the primary endpoint in the determination of toxicity and have not documented perturbations in reproduction, biochemical changes, susceptibility to environmental stress, immune function, or behavior. Furthermore, the test conditions used in many studies varied and may have contributed to apparent differences in the susceptibility of birds to toxicants (Tucker and Leitzke, 1979). The relative sensitivity of passerines to pesticides needs to be examined using a panel of endpoints with rigorously controlled experimental conditions before accurate statements regarding species sensitivity can be made.

Geographical distribution, reproductive rate, and the presence of a large data base (ecology, breeding biology, and toxicity data) all must be considered when selecting a bird species for toxicity testing. The house sparrow and starling have widespread distribution, high reproductive rates, and there is a wealth of information regarding their ecology, breeding biology, and response to pesticides. Because of these factors, the house sparrow and starling are well suited for toxicity testing.

Conclusion

In summary, the starling may be an appropriate Passerine species to use for testing the effects of pesticides on free-ranging birds. The starling is particularly well suited for nest box studies. The starling satisfies nearly all of the criteria used in the selection of a bird for testing the toxicity of pesticides under actual environmental conditions. The starling is found throughout North America, nests in agricultural areas, forages on ground-dwelling invertebrates during the breeding season, is economically important, readily utilizes nest boxes, is tolerant of human activity, has a high potential for exposure to agricultural chemicals by a variety of routes, does not require the acquisition of federal permits to study, occurs in large populations, and there is minimal opposition to its use for toxicity testing. Presently no other Passerine is as feasible for use in nest box studies as is the starling.

The only substantial reason for not using adult starlings for toxicity testing is their apparent resistance to pesticides. However, nestling starlings may be more sensitive to pesticides than adult starlings and therefore may be a more useful test organism. Also, through the use of alternative endpoints, more information on comparative biochemistry, and through pesticide studies using multiple Passerine species, the toxicity data obtained from starling nest box studies may be more accurately extrapolated to other Passerines.

APPENDIX 2

A SUMMARY OF THE BREEDING BIOLOGY OF THE STARLING

1.0 General Description

The European starling (Sturnus vulgaris) is a medium-sized bird which is about 20 cm long and usually weighs 75-100 g. The starling has a rather stocky build and the plumage is almost entirely dark. The wings are well suited for fast flight as they are relatively long and pointed. The starling spends much of its time on the gound foraging for food. Its powerful legs and strong pointed bill are well adapted for this purpose (Feare, 1984; Tinbergen, 1981).

During the fall and winter months starlings congregate in large communal roosts which can exceed 50,000 birds (Feare, 1984). During the breeding season starlings utilize small cavities as nesting sites (Kessel, 1957). A nest cavity may be located in the hollow of a tree, under the eaves of a roof, or in practically any other natural or man-made cavity. The starling is one of the most successful colonizing species of birds. Following its introduction in New York City in 1890, its numbers have increased to over 200 million. The starling's success has been attributed to its ability to readily adapt to a wide variety of food sources and to thrive in agricultural areas (Feare, 1984).

2.0 Geographical Distribution

The European starling became established in North America through introductions made in New York City in 1890 and 1891. Since then their range has expanded so that they are common from Canada and southeastern Alaska to the sub-tropics of Mexico. Starlings (Sturnus spp.) are found throughout Europe, western and central Asia, New Zealand, and parts of Australia and Africa (Feare 1984).

3.0 Breeding Biology

3.1 Nest Site Selection

Starlings will begin nest site selection activities in late fall and early winter. Kessel (1957) observed starlings visiting nesting sites in October and November and later found starlings roosting in the nest sites during December. Similar behavior of starlings was reported by Flux and Flux (1981) in New Zealand. Starlings were observed visiting nest sites at dawn and in the

evening throughout the year (except in December and January; post-breeding season in the southern hemisphere). Starlings generally select a small cavity or crevice in which to build a nest. However, if nesting sites are scarce, they may nest in rock piles, in nests contructed by other birds, on the ground, or in the branches of trees or shrubs (Kessel, 1957).

3.2 Mate Pairing

The male starling selects a nesting site, establishes and guards his nesting territory (generally an area of 0.5 m - 10.0 m surrounding the nesting site). Visual and auditory displays are used to attract females (Feare, 1984). Nest building activities are initiated by the male as an additional means of attracting a mate. Starlings are typically monogamous although cases of polygamy have been reported (Kessel, 1957)

3.3 Nest Building

After the pair has been established, nest building activities increase, with a peak in the activity occurring one week prior to egg-laying. As the date of egg laying approaches, the male provides fewer materials to the nest while the female dominates in nest building (Kessel, 1957, Feare, 1984). The completed nest is composed primarily of grassy material and frequently has a well developed nest cup approximately five cm across by four cm in depth. The nest cup is often lined with feathers or other insulating materials (Kessel, 1957). Starlings do not always produce nests with well developed cups prior to the laying of eggs in a nest; Kendall et al., (1988a) observed eggs in nest boxes which contained only a random assortment of nest building materials. Nest building efforts may continue after the laying of eggs (Kessel, 1957).

3.4 Copulation

Copulation can be observed at almost any time during the year. In North America copulation occurs with increasing frequency in March and continues throughout the breeding season. The male may display preliminary behavior, but the female generally initiates copulation during the breeding season. Copulation can occur on the ground, roofs, tree branches, or other similar sites (Kessel, 1957).

3.5 Egg Laying

Eggs are laid in early March to mid-June, depending on geographic location. The onset of egg laying is synchronous for a population of starlings living within a given geographical location. Kessel (1957) found that the date of egg laying for a majority of starlings using nest boxes in Ithaca, N.Y., was within a four day period. Flux and Flux (1981) reported a

similar synchronous date of egg laying from their study population; the date of egg laying exhibited a standard deviation of 1.94 days around the mean.

Following the laying of the first egg, the female lays one egg each consecutive day until the clutch is complete. Clutch sizes (for first clutches of mature birds) vary from three to five eggs with mean values of 4.9 (North America; Kessel, 1957), 4.77 (New Zealand; Flux and Flux, 1981), and 4.48-4.96 (Europe; Feare, 1984). Intermediate and second brood clutch sizes are generally smaller than those found in the first clutch (Feare, 1984).

3.6 Number of Clutches

Starlings will often lay two clutches during a breeding season. These second clutches are usually smaller than first clutches and less productive (Feare, 1984).

3.7 Nest Desertion

If the female deserts the nest or is killed during egg laying or incubation, the male will toss out the eggs within 36 hours and renew his courtship activities. Little is known about loss of the male during this same period (Kessel, 1957).

3.8 Incubation

Incubation usually begins after the last egg has been laid. Both the male and female adult share in incubation during the day, whereas, at night the female remains on the nest and incubates the eggs until early morning when she is relieved by the male. Eggs almost always hatch on the 12th day of incubation (Kluyver, 1933; Kessel, 1957; Kendall et al., 1988a). The female broods the nestlings during the night and is assisted in brooding during the day by the male. The adults continue to brood the nestlings until they are between 8 and 12 days of age (Kessel, 1957).

3.9 Care of Nestlings

In general, both adults take part in the feeding of the young. However, the female often makes more foraging sorties than the male and in some instances the male does not participate in feeding of the young (Kessel, 1957). Foraging decisions and food selection for the young have been extensively studied by

Tinbergen (1981) and Feare (1984). Tinbergen has shown that nestling dietary requirements vary with age and are an integral aspect of the parental decision-making process when foraging. Food types brought to the young also vary with clutch size, time of day, prey availability, and location of nest (Feare, 1984; Moore, 1986).

3.10 Nestlings

The average brood size for starlings over a four year period in Ithaca, N.Y., was 3.9 young for 304 broods (Kessel, 1957). Flux and Flux (1981) showed a range of 1.2 - 2.7 12-day-old chicks per nest, with an overall average of 1.92 chicks per nest. Feare (1984) reports that hatching success rates for first broods range from 73% to 91%. He cites a study by Korpimaki (1978) which found that the highest hatching success rate occurred in clutches of five or six eggs (85% to 90%). At hatching, the nestlings weigh about six g., are naked, have their eyes closed, and are completely helpless. Their eyes open on about the seventh day. The nestlings grow rapidly and by about the tenth day they have nearly attained the weight of an adult starling (70 g) (Kessel, 1957). The nestlings are able to fledge by 17 -19 days post-hatching but almost always fledge around 21 days of age (Feare, 1984). At fledging, the young are almost fully feathered and can fly well. About 85% to 90% of first brood young survive to this age (Kessel, 1957; Feare, 1984). The young of second broods are more irregular in their development, weigh less at fledging, and have lower fledging rates than the young of first broods (Kessel, 1957).

The fledglings usually remain in the vicinity of the nest and have been observed following the adult female for a period of 10 to 12 days (Feare, 1984). This time in the birds life is perhaps the most hazardous. Mortality rates among first-year birds are in the range of 56% to 76% (Kessel, 1957; Coulson, 1960; Feare, 1984). Female starlings are able to breed after one year, while the males frequently do not breed before two years of age. First year females have a smaller clutch size than females which are two to five years of age. There is generally a peak in productivity at the age of three to five years, following which the birds produce smaller clutches and often have higher mortality rates (Kessel, 1957).

4.0 Mortality Rates

Annual mortality estimates from ringed-bird recovery data in Europe, the United Kingdom, and in the United States have shown mortality rates of first year birds to be between 56% - 73%, while those of older birds ranges from 33% - 68%. climatic, agricultural, and human population differences may have an effect

on these figures, but currently the data are not conclusive (Feare, 1984). In the U.S., annual mortality rates for females are generally higher than those observed in males (Frankhauser, 1971).

5.0 Longevity

The average life span of a starling is between 12 - 18 months. Birds which have lived for 12 months can be expected to survive for another 12 -18 months. The maximum age recorded for a starling is 16 years in Britain and 17 years 8 months in the U.S. (Feare, 1984).

6.0 Feeding Ecology

Starlings are primarily grassland feeders. They utilize a wide range of food materials and studies have shown that their diets vary geographically, seasonally, and even daily (Feare, 1984). They are considered omnivorous, but because they are unable to digest large quantities of cellulose their consumption of plant material is usually restricted to seeds and fruits. In urban areas, household garbage often comprises a large portion of their diet. During the breeding season, their diet consists almost entirely of invertebrates obtained from the upper few centimeters of soil (Feare, 1984). Nestlings are fed a wide variety of invertebrates. The type of prey brought to them depends on prey availability, nutrient content of the food, and the state of hunger of the nestlings. Kluyver (1933) identified over 230 species of invertebrates eaten by starling nestlings including earthworms, snails, woodlice, centipedes, millipedes, grasshoppers, beetles, ants, flies and spiders. In most geographical areas, the two food items most often delivered to nestlings are larvae of the insect orders Coleoptera (beetles) and Lepidoptera (butterflies and moths) (Feare, 1984).